A SURVEY OF THE TAPEWORM FAUNA OF THE COWNOSE RAY, *RHINOPTERA BONASUS,* FROM THE NORTHERN GULF OF MEXICO WITH COMMENTS ON INTERMEDIATE HOSTS

BY

Garrett Call

Submitted to the Department of Ecology and Evolutionary Biology
and the faculty of the Graduate School of the University of Kansas
in partial fulfillment of the requirements for the degree of
Master's of Arts

_____
Chairperson

Committee members _____

_____

Date defended: _____

The Thesis Committee for Garrett Call certifies
that this is the approved version of the following thesis:

A SURVEY OF THE TAPEWORM FAUNA OF THE COWNOSE RAY, *RHINOPTERA BONASUS,* FROM THE NORTHERN GULF OF MEXICO WITH COMMENTS ON INTERMEDIATE HOSTS

Committee:

_____
Chairperson

_____

_____

Date approved: _____

AUTHOR'S DISCLAIMER

All taxonomic actions in this work are hereby disclaimed for nomenclatural purposes, as recommended in Article 8 of the International Code of Zoological Nomenclature (Ride et al., 1999)

Acknowledgments

I wish to express my sincere gratitude to my advisor Dr. Kirsten Jensen whose encouragement, patience, generosity, flexibility, and support made this project possible. Her enthusiastic instruction, intellectual curiosity, and scientific rigor are traits that I hope to emulate in the future. My gratitude also extends to Dr. Stephen Bullard of the Gulf Coast Research Laboratory whose collecting efforts were absolutely critical to this project and whose instruction, expertise, and humor in the field (especially when collecting cownose rays) and the laboratory will be forever appreciated. A special thanks goes to Dr. Robin Overstreet, who permitted the use of the facilities at the Gulf Coast Research Laboratory. It was always a treat when Dr. Overstreet and asked me how things were going after many hours of dissection of *Donax variabilis*. I would like to thank Dr. Richard Heard for help with the identification of various molluscs. Also thanks to my committee members, Dr. Daphne Fautin and Dr. Ed Wiley, for their comments and helpful insights.

I am grateful to the people who helped in the collection and dissection of *D. variabilis*: Dr. Pavel Nikolov, Hannah Owens, Kendra Koch, Carrie Fyler, and Maria Pickering. The molecular work could not have been conducted without the help and expertise of Jenny Kuszak (Veterinary Diagnostics Center, Lincoln, Nebraska, U.S.A.) and Mike Grose (KU Molecular Laboratory). A special thanks also goes to Dr. Bruce Cutler for his knowledge and ability to coax the scanning electron microscope into working order. I would also like to extend a sincere thanks to Dr. Janine Caira (University of Connecticut) for discussions of the genus *Duplicibothrium*.

My family has always encouraged me in all my life's choices. I am grateful to my parents, Patrick and Karen Call, grandparents Roy and Margaret Chatham, and Uncle Bill and Aunt Rita Blauvelt for their curiosity and support. Shortly before embarking on this project, I met Sarah Sedlock, and at the end of this project she is my wife. Her unconditional love, support, and encouragement of my interests and work have been a continual source of strength.

This project was funded by a Kansas NSF EPSCoR First Award to K. Jensen, University of Kansas.

Table of Contents

List of Tables

List of Figures

Abstract

This survey determined the cestode diversity parasitizing the spiral intestine of the cownose ray, *Rhinoptera bonasus* (Mitchill), in the northern Gulf of Mexico. Twelve cownose rays were collected near barrier islands off Ocean Springs, Mississippi, U.S.A. Thirteen species were identified. Eight were previously known; and the geographic distribution of seven expanded to include the Gulf of Mexico. *Duplicibothrium minutum* is redescribed. Two species each of *Duplicibothrium* and *Eniochobothrium* and one *Polypocephalus* species are described. Nineteen species are now known to parasitize *R. bonasus* throughout its range.

The coquina clam, *Donax variabilis* (Say), inhabiting the swash zones of the barrier islands that *R. bonasus* frequents, were examined for larval tapeworms. Examination of 915 clams collected revealed two types of larvae. These larvae were identified as *Rhodobothrium paucitesticulare* and *D. minutum*, parasitizing *R. bonasus* as adults, using morphology and molecular sequence data.

Prevalence and intensity of adults and larvae are presented.

TAPEWORM FAUNA OF *RHINOPTERA BONASUS*

Introduction

Tapeworms, a diverse, monophyletic group of parasitic flatworms, belong to the class Cestoda of the phylum Platyhelminthes. Eight thousand described species in approximately 650 genera parasitize a variety of vertebrate hosts (Caira and Littlewood, 2001). These numbers represent underestimates of the actual diversity, because the cestode fauna has yet to be examined for many vertebrates. About 800 described species from five orders are known from the sharks and rays (Elasmobranchii), and, as for most vertebrates, the diversity within this group is particularly underestimated (see Caira and Healy, 2004). This survey focuses on a small portion of this underestimated diversity within the elasmobranchs. The cestodes identified in this survey infect the spiral intestine of the cownose ray, *Rhinoptera bonasus* (Mitchill) (Myliobatiformes, Rhinopteridae).

The family Rhinopteridae is monotypic, with only a single genus recognized, and *Rhinoptera bonasus* is one of 11 nominal species in the genus (Compagno, 1999), of which five are considered valid by Schwartz (1990). It is the only species of *Rhinoptera* to inhabit the northern Gulf of Mexico. The other valid species in this family are: the Javanese cownose ray, *Rhinoptera javanica* Müller and Henle; the Lusitanian cownose ray, *Rhinoptera marginata* (Geoffrey Saint-Hilaire); the Australian cownose ray, *Rhinoptera neglecta* Ogilby; and the Pacific cownose ray, *Rhinoptera steindachneri* Evermann and Jenkins. *Rhinoptera bonasus* ranges throughout the warm coastal waters of the western Atlantic Ocean from southern New

England to southern Brazil and the eastern Atlantic Ocean off Mauritania, Senegal, and Guinea (McEachran and Seret, 1990; Neer and Thompson, 2005; Schwartz, 1990).

Rhinoptera bonasus is semi-pelagic and gregarious, often forming schools numbering in the thousands (Blaylock, 1989; Neer and Thompson, 2005; Schwartz, 1990). These schools reportedly migrate south from Chesapeake Bay to North Carolina or as far south as northern South America during the winter (in the northern hemisphere) seeking warmer water (Rogers et al., 1990; Smith and Merriner, 1987). Schwartz (1965) suggested the migration of cownose rays starts in the autumn off the west coast of Florida with rays moving along the coastline to the Yucatan Peninsula. In addition to these migratory patterns, Smith and Merriner (1987) suggested that semi-isolated populations exist in Brazil, northeastern South America, the Gulf of Mexico, and the east coast of the United States. Neer and Thompson (2005) examined rays in the Gulf of Mexico and compared their age and growth to those cownose rays studied by Smith and Merriner (1987) in the Chesapeake Bay, Virginia, U.S.A. Near and Thompson (2005) found that cownose rays in the Gulf of Mexico achieve maturity at an earlier age and live longer than those in Chesapeake Bay and may have a slightly longer pupping season.

Prior to this study, 13 species of tapeworms, representing four of the five orders known to parasitize elasmobranchs, had been reported to parasitize *Rhinoptera bonasus* throughout its range (Table 1). These reports consisted of records of single to a few tapeworm species from *R. bonasus* from localities on the east coast of the

United States, the Gulf of Venezuela, the coastal waters of Senegal, and the northern Gulf of Mexico. Until this study, no complete faunal survey has been conducted on the cestodes of *R. bonasus* from a single locality. The primary aim of this study was to determine the diversity of tapeworms parasitizing *R. bonasus* in the northern Gulf of Mexico, a region from which only two species had previously been reported (see Table 1).

In addition to the faunal survey, this study aimed to determine identities of larval cestodes that potentially parasitize *R. bonasus* as adults, using morphology and molecular techniques. This secondary aim is treated in detail in the second part of this thesis.

Table 1. Tapeworm species reported from *Rhinoptera bonasus* at the inception of this study.

Species	Type Locality	Other Localities	References
Order Trypanorhyncha			
Mecistobothrium brevispine (Linton, 1897) Campbell & Carvajal, 1975	Woods Hole, MA, U.S.A.	Chesapeake Bay, VA, U.S.A.	Linton (1897); Campbell & Carvajal (1975)
Nybelinia sp.		Woods Hole, MA, U.S.A.	Linton (1897) (as *Tetrarhynchus robustum*); Palm (2004)
Rhinoptericola megacantha Carvajal & Campbell, 1975	Chesapeake Bay, VA, U.S.A.	Gulf of Venezuela, Venezuela	Carvajal & Campbell (1975); Mayes & Brooks (1981)
Zygorynchus sp.		Woods Hole, MA, U.S.A.	Linton (1897, 1901); Palm (2004)
Order Diphyllidea			
Echinobothrium bonasum Williams & Campbell, 1980	Chesapeake Bay, VA, U.S.A.	Sakonnet Point, RI, U.S.A.	Williams & Campbell (1980)
Order Tetraphyllidea			
Dioecotaenia campbelli Mayes & Brooks, 1981	Gulf of Venezuela, Venezuela		Mayes & Brooks (1981)
Dioecotaenia cancellata (Linton, 1890) Schmidt, 1969	Chesapeake Bay, MA, U.S.A.	Woods Hole, MA, U.S.A.; Chesapeake Bay, VA, U.S.A.	Linton (1890); Schmidt (1969); Olson et al. (1999)
Duplicibothrium minutum Williams & Campbell, 1978	Chesapeake Bay, VA, U.S.A.	Sakonnet Point, RI, U.S.A.; Ocean Springs, MS, U.S.A.; E. Atlantic Ocean, Senegal	Williams & Campbell (1978); Olson et al. (1999); Healy (2006)
Glyphobothrium zwerneri Williams & Campbell, 1977	Chesapeake Bay, VA, U.S.A.		Williams & Campbell (1977)
Rhodobothrium paucitesticulare Mayes & Brooks, 1981	Gulf of Venezuela, Venezuela	Ocean Springs, MS, U.S.A	Mayes & Brooks (1981); Olson et al. (1999) (as *Rhodobothrium* sp.)
Order Lecanicephalidea			
Tylocephalum bonasum Campbell & Williams, 1984	Sakonnet Point, RI, U.S.A.		Campbell & Williams (1984)
Tylocephalum brooksi Ivanov & Campbell, 2000[1]	Gulf of Venezuela, Venezuela		Ivanov & Campbell (2000)
Tylocephalum pingue (Linton, 1890) Campbell & Williams, 1984	Woods Hole, MA, U.S.A.	Chesapeake Bay, VA, U.S.A.; Sakonnet Point, RI, U.S.A.	Linton (1890); Campbell & Williams (1984)

[1] The specimens, reported as *Tylocephalum* sp. by Mayes and Brooks (1981), are likely to constitute the material used to describe *T. brooksi;* however, verification is required.

Materials and Methods

Collections

Specimens of *Rhinoptera bonasus* were caught by treble hook and line or using a spear gun. Twelve specimens (one male, four females, one immature, and six of unrecorded sex) were caught during the months of June, August, and November 2005, and March, April, August, and October 2006. Cownose rays were caught at: Ship Island, Horn Island, and Chandeleur Islands off Ocean Springs, Mississippi, U.S.A. Necropsies were performed on each ray in the field; the spiral intestine was removed and opened with a longitudinal incision. In all cases except one, the spiral intestine was placed in 10% seawater-buffered formalin and agitated for approximately 5 min. After 2-7 days the spiral intestines were transferred to 70% ethanol for storage. Cestodes present in the intestine were fixed *in situ* or removed prior to fixation and fixed separately. Specimens for morphological examination using light and scanning electron microscopy (SEM) were placed in 10% seawater-buffered formalin and gently shaken. These cestodes were later transferred to 70% ethanol for storage. A single spiral intestine was placed in 95% ethanol and agitated for approximately 5 min, then stored at subzero temperatures in 95% ethanol for molecular work. Cestodes, fixed separately for molecular work, were placed in 95% ethanol. These specimens were also stored at subzero temperatures after return to the laboratory.

Specimen preparation

Cestodes prepared as whole mounts were hydrated in distilled water, stained with Delafield's hematoxylin, differentiated in tap water, destained in 70% acid ethanol, alkalinized in 70% basic ethanol, dehydrated in 95% and 100% ethanol, cleared in methyl salicylate, and mounted in Canada balsam on glass slides. Cestode egg mounts were prepared as follows. Gravid proglottids were separated from the strobila, which was retained as a voucher. The proglottids were placed in a lactophenol/70% ethanol mixture, and left uncovered under an exhaust hood for 4-6 h. The proglottids were placed on a glass slide in a drop of lactophenol and teased open with insect pins to release the eggs. The larger pieces of the proglottids were removed, and the remainder placed under a coverslip and sealed with nail polish.

Cestodes prepared as histological sections were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraplast (Tyco Healthcare Group LP, Mansfield, Massachusetts). Sections 7 μm thick were cut using an Olympus CUT4060 retracting rotary microtome. Sections were affixed to glass slides with a 3% sodium silicate solution, hydrated in a graded ethanol series, stained in Delafield's hematoxylin, counterstained in eosin, differentiated in Scott's solution, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Specimens prepared for scanning electron microscopy (SEM) were hydrated in distilled water, placed in 1% osmium tetroxide overnight, dehydrated in a graded ethanol series, dried using hexamethyldisilizane (Electron Microscopy Services, Fort Washington, Pennsylvania), mounted on aluminum stubs using double-sided carbon

tape, and sputter-coated with approximately 35 nm of gold. Scanning electron microscopy was performed with a Zeiss LEO 1550 field emission scanning electron microscope. Measurements were taken using a computer video imaging system consisting of a Leica Firecam DFC 320/480 digital camera mounted on a Zeiss Axioscop using the image analysis program Openlab Demo Version 4.0.4. Line drawings were made using a camera lucida. All measurements in the text are given in micrometers (μm) unless stated otherwise. Measurement are given as the range, followed in parentheses by the mean, standard deviation, number of worms measured, and number of measurements if more than one measurement was taken per worm. Due to the thin region attaching the scolex to the trough in species of *Eniochobothrium*, the scolex easily detaches from the strobila. Total lengths of *Eniochobothrium* species described are presented for both intact worms and those that had lost their scolices. The hook formula used herein follows that of Tyler (2006). Ecological terms used are defined as follows: 1) Prevalence is defined here as the number of hosts infected by a parasite species divided by the number of hosts sampled (expressed as a percentage) 2) Intensity is defined here as the number of parasites of a species in a infected host individual 3) Infracommunity is defined as the assemblage of parasite species in a host individual. Museums abbreviations used as follows: HWML, Harold W. Manter Laboratory, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A.; KUNHM, University of Kansas Natural History Museum, Division of Invertebrate Zoology, University of Kansas, Lawrence, Kansas, U.S.A.; LRP, Lawrence R. Penner Parasitology Collection, Department of Ecology

and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, U.S.A.; USNPC, United States National Parasite Collection, Beltsville, Maryland, U.S.A. Host classifications follow Nelson et al. (2004).

Results

Tapeworm species recovered from the 12 rays consisted of 13 species representing four of the five orders known to parasitize elasmobranchs. Of these, eight species were previously described (i.e., *Mecistobothrium brevispine*, *Rhinoptericola megacantha*, *Echinobothrium bonasum*, *Echinobothrium fautleyae*, *Dioecotaenia cancellata*, *Rhodobothrium paucitesticulare*, *Duplicibothrium minutum*, and *Tylocephalum bonasum*) and five species are new to science. Each of the previously described species is identified and distinguished; new morphological information for some is presented. *Duplicibothrium minutum* is redescribed and the descriptions of five new species follows. Prevalence and intensity of each species were recorded.

Prevalence ranged from 8-92%; thus no single species parasitized every host individual (Table 3). Two species, *E. fautleyae* and *D. minutum.* infected 11 of the 12 rays (Table 3, 4) whereas one species, *E. bonasum*, was found in a single ray. *Echinobothrium bonasum* also had the lowest intensity with only one specimen found, whereas four species, *E. fautleyae, D. minutum, D. karenae,* and *E. overstreeti*, had intensities of greater than 100 worms (Table 3). The highest number of species found in a single host was eight and the lowest was three (Table 4).

Table 2. Tapeworm species reported from *Rhinoptera bonasus* at the conclusion of this study. (Note: * indicates species collected in this study)

Species	Type Locality	Other Localities	References
Order Trypanorhyncha			
**Mecistobothrium brevispine* (Linton, 1897) Campbell & Carvajal, 1975	Woods Hole, MA, U.S.A.	Chesapeake Bay, VA, U.S.A.; Ship Island, MS, Gulf of Mexico, U.S.A.	Linton (1897); Campbell & Carvajal (1975); this study
Nybelinia sp.		Woods Hole, MA, U.S.A.	Linton (1897) (as *Tetrarhynchus robustum*); Palm (2004)
**Rhinoptericola megacantha* Carvajal & Campbell, 1975	Chesapeake Bay, VA, U.S.A.	Gulf of Venezuela, Venezuela; Ship & Horn Island, MS, Gulf of Mexico, U.S.A.	Carvajal & Campbell (1975); Mayes & Brooks (1981); this study
Zygorynchus sp.		Woods Hole, MA, U.S.A.	Linton (1897, 1901); Palm (2004)
Order Diphyllidea			
**Echinobothrium bonasum* Williams & Campbell, 1980	Chesapeake Bay, VA, U.S.A.	Sakonnet Point, RI, U.S.A.; Horn Island, MS, Gulf of Mexico, U.S.A.	Williams & Campbell (1980); this study
**Echinobothrium fautleyae* Tyler & Caira, 1999	Puertecitos, Gulf of California, Mexico	Bahia de Los Angeles, Santa Rosalia, Loreto, Punta Arenas, Gulf of California, Mexico; Puerto Viejo, Baja California Sur, Mexico; Ship & Horn Island, MS, Gulf of Mexico, U.S.A, Chandeleur Island, LA, Gulf of Mexico U.S. A.	Tyler & Caira (1999); this study
Order Tetraphyllidea			
Dioecotaenia campbelli Mayes & Brooks, 1981	Gulf of Venezuela, Venezuela		Mayes & Brooks (1981)
**Dioecotaenia cancellata* (Linton, 1890) Schmidt, 1969	Chesapeake Bay, MA, U.S.A.	Woods Hole, MA, U.S.A.; Chesapeake Bay, VA, U.S.A.	Linton (1890); Schmidt (1969); Olson et al. (1999); this study

Table 2 Con't.

Species	Type Locality	Other Localities	References
Order Tetraphyllidea			
**Duplicibothrium minutum* Williams & Campbell, 1978	Chesapeake Bay, VA, U.S.A.	Sakonnet Point, RI, U.S.A.; E. Atlantic Ocean Senegal; Ocean Springs, MS, U.S.A.; Ship & Horn Island, MS, Gulf of Mexico U.S.A.; Chandeleur Island, LA, Gulf of Mexico, U.S.A.	Williams & Campbell (1978); Olson et al. (1999); Healy (2006); this study
**Duplicibothrium karenae* n. sp.	Horn Island, MS, Gulf of Mexico, U.S.A.	Ship Island, MS, Gulf of Mexico, U.S.A.	this study
**Duplicibothrium mergacephalum* n. sp.	Horn Island, MS, Gulf of Mexico, U.S.A.		this study
Glyphobothrium zwerneri Williams & Campbell, 1977	Chesapeake Bay, VA, U.S.A.		Williams & Campbell (1977)
**Rhodobothrium paucitesticulare* Mayes & Brooks, 1981	Gulf of Venezuela, Venezuela	Ocean Springs, MS, U.S.A	Mayes & Brooks (1981); Olson et al. (1999) (as *Rhodobothrium* sp.); this study
Order Lecanicephalidea			
**Eniochobothrium overstreeti* n. sp.	Horn Island, MS, Gulf of Mexico, U.S.A.	Ship Island, MS, Gulf of Mexico, U.S.A.	this study
**Eniochobothrium sedlockae* n. sp.	Horn Island, MS, Gulf of Mexico, U.S.A.	Chandeleur Islands, LA, Gulf of Mexico, U.S.A.	this study
**Polypocephalus patricki* n. sp.	Ship Island, MS, Gulf of Mexico, U.S.A.	Horn Island, MS, Gulf of Mexico, U.S.A.	this study
**Tylocephalum bonasum* Campbell & Williams, 1984	Sakonnet Point, RI, U.S.A.	Ship Island, MS, Gulf of Mexico, U.S.A.	Campbell & Williams (1984); this study
Tylocephalum brooksi Ivanov & Campbell, 2000	Gulf of Venezuela, Venezuela		Ivanov & Campbell (2000)
Tylocephalum pingue (Linton, 1890) Campbell & Williams, 1984	Woods Hole, MA, U.S.A.	Chesapeake Bay, VA, U.S.A.; Sakonnet Point, RI, U.S.A.	Linton (1890); Campbell & Williams (1984)

Table 3. Prevalence and intensity of species collected in this study.

Species	**Prevalence**	**Estimated Intensity**
Mecistobothrium brevispine	8% (1 of 12)	3
Rhinoptericola megacantha	50% (6 of 12)	2-8
Echinobothrium bonasum	8% (1 of 12)	1
Echinobothrium fautleyae	75% (9 of 12)	1->100
Dioecotaenia cancellata	8% (1 of 12)	>20
Duplicibothrium minutum	92% (11 of 12)	5->100
Duplicibothrium karenae n. sp.	33% (4 of 12)	1->100
Duplicibothrium mergacephalum n. sp.	25% (4 of 12)	1-48
Rhodobothrium paucitesticulare	50% (6 of 12)	1-28
Eniochobothrium overstreeti n. sp.	58% (7 of 12)	9->100
Eniochobothrium sedlockae n. sp.	33% (4 of 12)	2-14
Polypocephalus patricki n. sp.	58% (7 of 12)	1-25
Tylocephalum bonasum	17% (2 of 12)	1-4

Table 4. Tapeworm infracommunity in each host specimen.

Specimen No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Species												
Mecistobothrium brevispine	x											
Rhinoptericola megacantha	x		x			x	x	x		x		
Echinobothrium bonasum						x						
Echinobothrium fautleyae	x	x	x	x	x	x	x	x		x	x	x
Dioecotaenia cancellata		x										
Duplicibothrium minutum	x	x	x		x	x	x	x	x	x	x	x
Duplicibothrium karenae n. sp.	x		x		x					x		
Duplicibothrium mergacephalum n. sp.						x	x	x		x		
Rhodobothrium paucitesticulare	x			x	x	x	x	x				
Eniochobothrium overstreeti n.sp.	x		x	x	x					x	x	x
Eniochobothrium sedlockae n. sp.					x		x		x	x		
Polypocephalus patricki n. sp.	x			x	x		x	x	x			x
Tylocephalum bonasum			x					x				

Previously Described Species

Mecistobothrium brevispine (Linton, 1897) Campbell and Carvajal, 1975

(Fig. 1)

Voucher specimens deposited: Two whole mounted vouchers (USNPC No. 00000).

The genus *Mecistobothrium* Heinz and Daily, 1974, a member of the family Eutetrarhynchidae, is characteristic in its possession of large bothria, a short pars vaginalis, and relatively short retracted bulbs (Palm, 2004). It shares features with the tentaculariid, *Heteronybelinia* Palm, 1999, from which it can be distinguished based on the presence of prebulbar organs, gland cells inside the bulbs, and the possession of two, rather than four bothria (Palm, 2004). In addition to *M. brevispine*, *Mecistobothrium* includes five species: *M. johnstonei* (Southwell, 1929) Beveridge and Campbell, 1998, from the dasyatid stingrays, *Pastinachus sephen* (Forsskål), and *Dasyatis kuhlii* (Müller and Henle); *M. myliobati* Heinz and Dailey, 1974, from *Myliobatis californica* Gill and *Urobatis halleri* Cooper;*M. pauciortesticulatum* Palm, 2004, from *Taeniura lymna* (Forsskål); and *M. penaeus* Feigenbaum, 1975, from the shrimp *Farfantepenaeus brasiliensis* (Latreille) and *F. duorarum* (Burkenroad) (see Palm 2004).

Identification of the specimens collected as part of this study as *M. brevispine* was based on the lack of a basal swelling, possession of two oval bothria, and the length of the scolex. *Mecistobothrium johnsonei* and *M. pauciortesticulatum* both possess a basal swelling. *Mecistobothrium myliobati* possesses a shorter scolex than

M. brevispine (490-830 vs. 1,382-1,581, respectively). *Mecistobothrium brevispine* is distinguished from *M. penaeus* based on the possession of oval bothria rather than triangular bothria and a shorter scolex. Palm (2004) considered *M. brevispine* to possess eight principal hooks ascending in a half spiral row. The specimens examined in this study were observed to possess a least nine principal hooks.

Mecistobothrium brevispine was present in one of the 12 cownose rays.

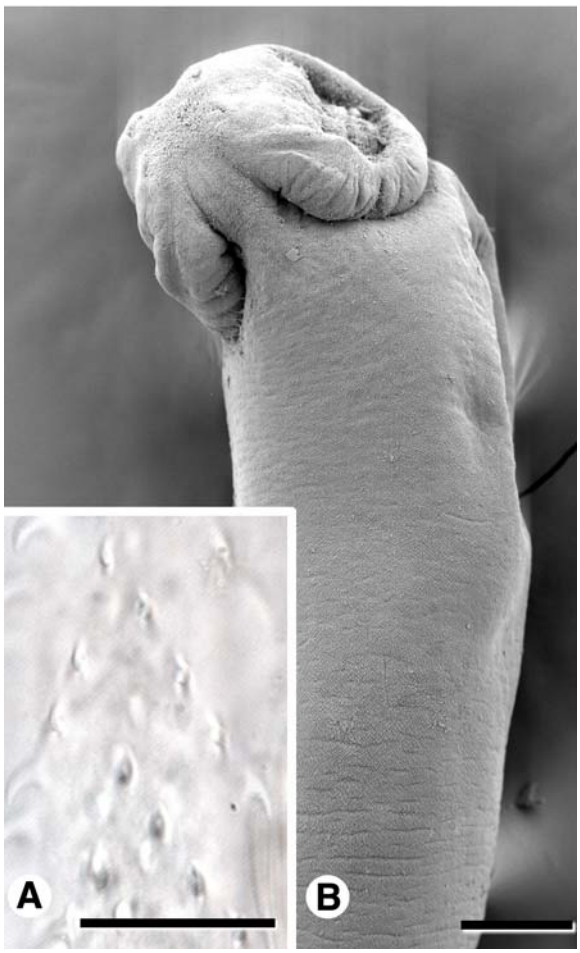

Figure 1. Light micrograph and scanning electron micrograph of *Mecistobothrium brevispine*. (A) External surface, metabasal armature. (B) Scolex. Scale bars: A, 20 µm; B, 100 µm.

Rhinoptericola megacantha Carvajal and Campbell, 1975

(Fig. 2)

Voucher specimens deposited: Two whole mounted vouchers (USNPC No. 00000).

According to Palm (2004), the family Rhinoptericolidae, to which *R. megacantha* belongs, includes three monotypic genera. Besides *R. megacantha,* they are *Shirleyrhynchus aetobatidis* (Shipley and Hornell, 1906) Beveridge and Campbell, 1998, and *Cetorhinicola acanthocapax* Beveridge and Campbell, 1988. The other two members parasitize hosts other than *R. bonasus* (i.e., myliobatid and dasyatid rays, and the Basking shark, *Cetorhinus maximus*, respectively).

Identification of *R. megacantha* was based on the possession of four well-separated bothria lacking bothrial pits and large uncinate hooks and falciform hooks present on the metabasal armature (Fig. 2A) as described by Carvajal and Campbell (1975). The collected specimens in this study possessed genital pores in the anterior third of mature proglottids and circumcortical vitellaria extending from the anterior margin of proglottid to the anterior margin of the ovary, consistent with the description of *R. megacantha*. *Rhinoptericola megacantha* differs from the other two members of the family based on, among other features, the presence of five principal hooks on the external surface, in contrast to eight principal hooks in *S. aetobatidis* and seven principal hooks in *C. acanthocapax*.

The eggs were described by Carvajal and Campbell (1975) as being 26 μm in diameter, but no illustrations were included. Illustrations of eggs are presented from specimens collected during this study (Fig. 2 C).

Rhinoptericola megacantha was present in six of the 12 cownose rays.

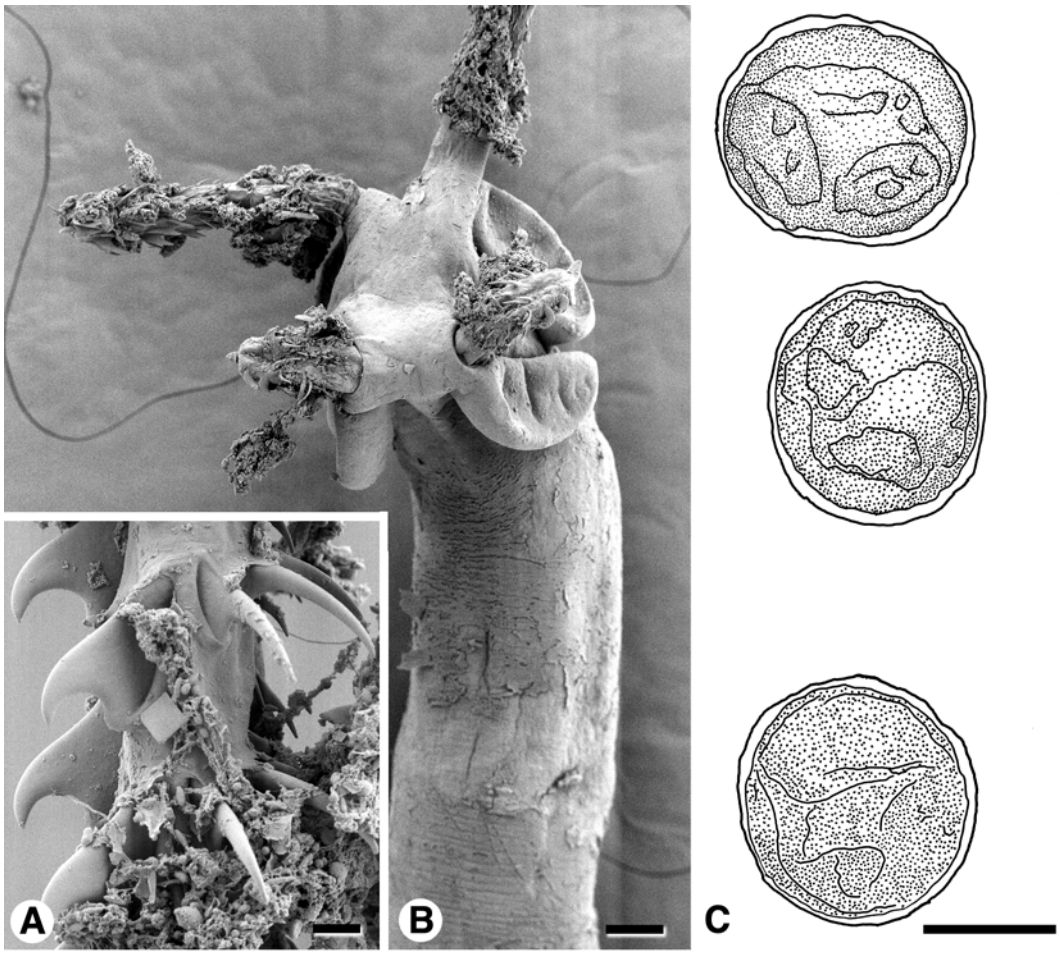

Figure 2. Scanning electron micrographs and line drawing of *Rhinoptericola megacantha*. (A) Internal surface, metabasal armature. (B) Scolex. (C) Eggs. Scale bars: A, C, 20 µm; B, 100 µm.

Echinobothrium bonasum Williams and Campbell, 1980

(Fig. 3 A-B)

Material examined: USNPC No. 75770 (holotype); USNPC Nos. 75771, 75772 (two paratypes).

Voucher specimens deposited: One whole mounted voucher (USNPC No. 00000).

A single specimen consistent with the original description of *E. bonasum* by Williams and Campbell (1980) was collected. It possessed the characteristic hook formula of {(12-14) 6/5 (12-14)}. All lateral hooklets were of equal length and no long lateral hooklets adjacent to the apical hook armature were observed (Fig. 3 B).

The hook formula distinguishes this species from all other species in the genus except *E. affine, E. harfordi,* and *E. fautleyae*. *Echinobothrium bonasum* possesses lateral hooklets arranged in a continuous row, as opposed to two groups of lateral hooklets in the former two species. *Echinobothrium bonasum* is distinguished from *E. fautleyae* based on having all lateral hooklets of relatively equal length, whereas the first and last hooklet of each row on *E. fautleyae* are almost twice the size of the others in the row.

Tyler (2006), in his monograph on the Diphyllidea, presented a modified description of *E. bonasum* based on the type material only. He had examined specimens of *Echinobothrium* from the type host, *R. bonasus*, from Ocean Springs, MS, U. S. A. and the east coast of the United States, but stated that the morphology from the newly collected specimens was not consistent with that of the type

specimens nor the original description of *E. bonasum*; consequently he did not include these specimens in his modified description of *E. bonasum*.

Echinobothrium bonasum was present in one of the 12 cownose rays.

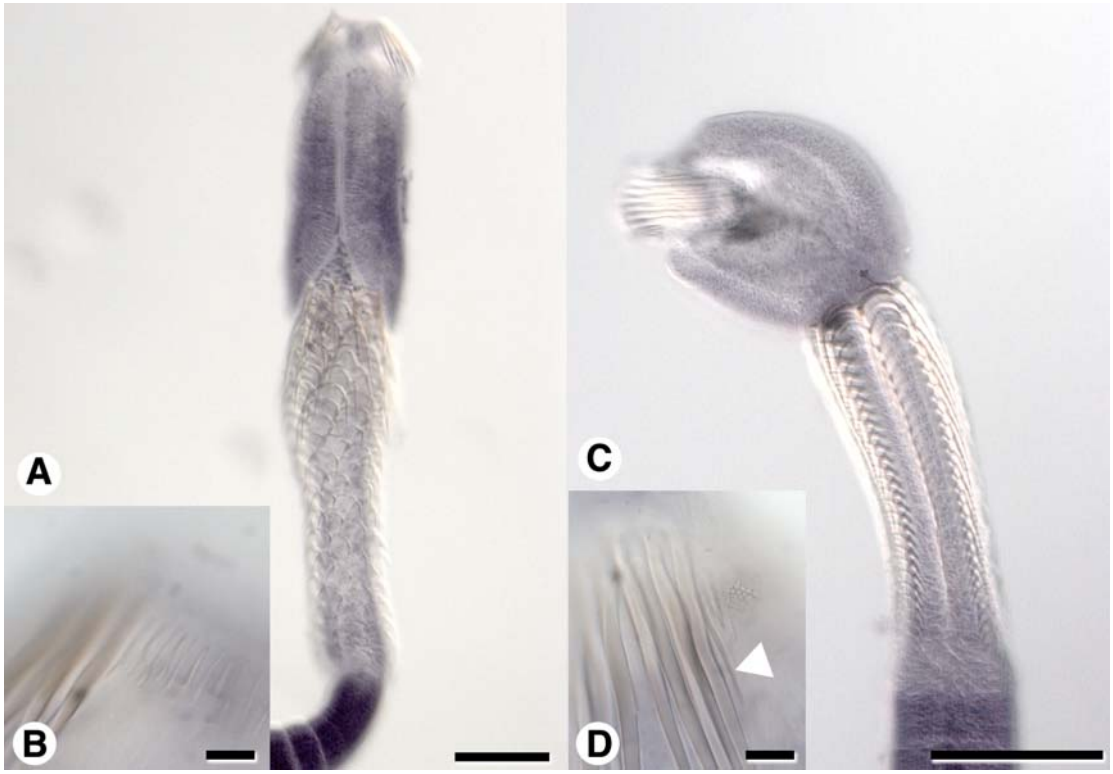

Figure 3. Light micrographs of *Echinobothrium bonasum.* (A) Scolex. (B) Lateral hooklets. Light micrographs of *Echinobothrium fautleyae*. (C) Scolex of *E. fautleyae*. (D) Lateral hooklets; arrow indicates longer lateral hooklet. Scale bars: A, C, 100 µm; B, D, 10 µm.

Echinobothrium fautleyae Tyler and Caira, 1999

(Figs. 3 C-D, 4)

Material examined: USNPC Nos. 88217, 88218, 88219 (seven paratypes).

Voucher specimens deposited: Two whole mounted vouchers (USNPC No. 00000).

Identification of specimens of *E. fautleyae* was based on a hook formula of {(11-12) 6/5 (11-12)} consistent with the original description of the species. The hook formula distinguishes *E. fautleyae* from all other species of *Echinobothrium* except *E. affine, E. harfordi,* and *E. bonasum*. *Echinobothrium fautleyae* differs from the first two species in its possession of lateral hooklets in a continuous row as opposed to two groups. The feature that distinguishes *E. fautleyae* from *E. bonasum* is that the first and last hooklet in each row are nearly twice as long as the others in the row, whereas in *E. bonasum* all lateral hooklets are of similar length.

Prior to this study, *E. fautleyae* had been reported from two hosts, *Rhinoptera steindachneri* and *Myliobatis californicus* Gill, from the eastern Pacific Ocean (Tyler, 2006). Tyler (2006) examined specimens collected from *R. bonasus* from the Gulf of Mexico and the east coast of the U.S.A. which were an intermediate in form between *E. bonasum* and *E. fautleyae*; these features were not articulated and it is unclear to which features Tyler is referring. The hook formula and the presence of the long lateral hooklets identify the collected specimens from *R. bonasus* as *E. fautleyae*. However, the specimens collected in this study possess a greater range in number of proglottids than was given in the original description (5-11 vs. 4-6).

Microthrix data obtained from collected specimens show pectinate spinitriches bearing three digits with the central digit longer than the others on the proximal bothrial surface (Fig. 4 B). This pattern is consistent with the proximal bothrial surface of the species.

Echinobothrium fautleyae was present in 11 of 12 rays.

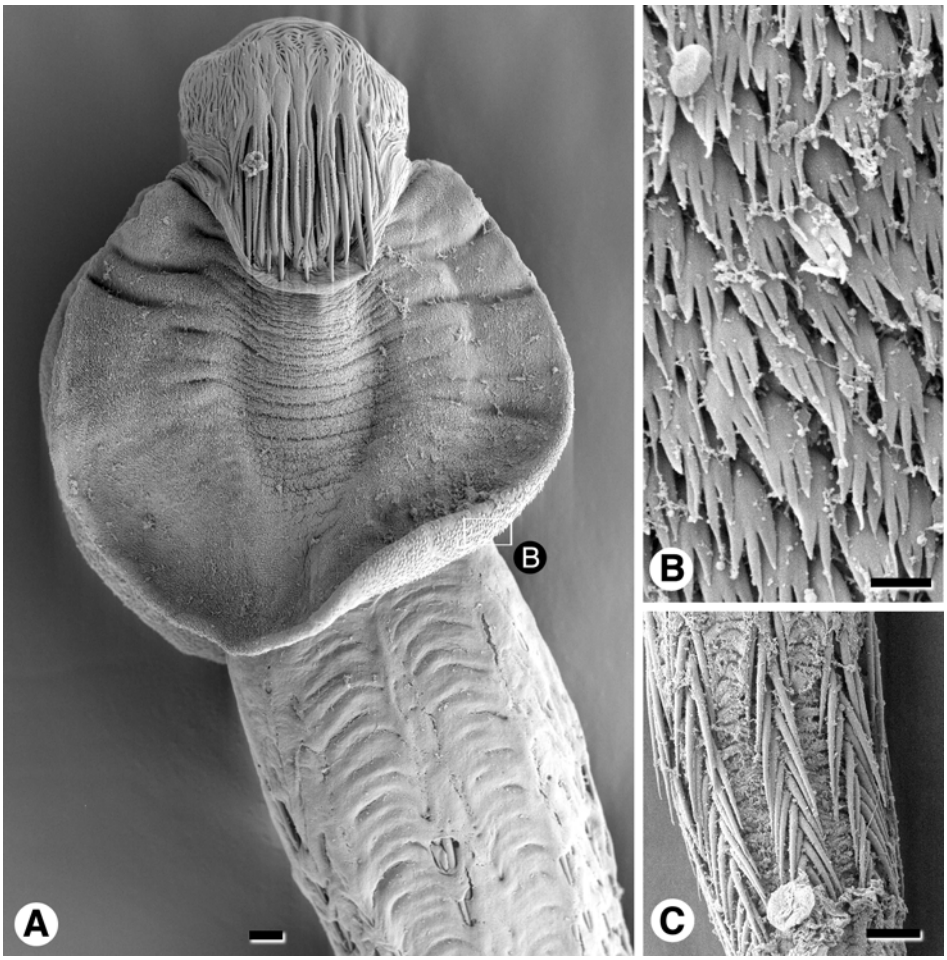

Figure 4. Scanning electron micrographs of *Echinobothrium fautleyae*. (A) Scolex; box indicates where Fig. 4B was taken. (B). Proximal bothrial surface. (C) Peduncle spines. Scale bars: A, 10 µm; B, 1 µm; C, 20 µm.

Dioecotaenia cancellata (Linton, 1890) Schmidt, 1969

(Figs. 6-7)

Material examined: *D. cancellata*: USNPC No. 71320 (eight vouchers); *D. campbelli*: USNPC No. 75719 (holotype), USNPC No. 75720 (allotype).

Voucher specimens deposited: Two whole mounted vouchers (USNPC No. 00000).

The genus *Dioecotaenia* is the only dioecious tetraphyllidean genus. Only male specimens were collected as part of this study. These specimens possessed 22 loculi per bothridium, i.e., median row of five loculi surrounded by 17 marginal loculi. Only two species have been described in this genus, *D. campbelli* and *D. cancellata*. Mayes and Brooks (1981) described *D. campbelli* as possessing 24 bothridial loculi, i.e., a median row of six loculi surrounded by 18 marginal loculi. Both Linton (1890) and Schmidt (1969) described *D. cancellata* as possessing 21 boththridial loculi, i.e., median row of five loculi surrounded by 16 marginal loculi. Selected type material of *D. campbelli* consistently possessed 24 loculi per bothridium (a median row of six surrounded by 18 marginal loculi) (Fig. 5). Voucher specimens of *D. cancellata* (USNPC No. 71320) exhibited a range of 20-22 loculi (Fig. 6), rather than a consistent 21 loculi as previously described. The only other features presented in the description of *D. campbelli* to distinguish it from *D. cancellata* are egg and embryo size. Because the specimens collected as part of the study were all males, these features could not be determined. Based on loculi number alone the specimens at hand are identified as *D. cancellata*.

Dioecotaenia cancellata was present in one of 12 cownose rays.

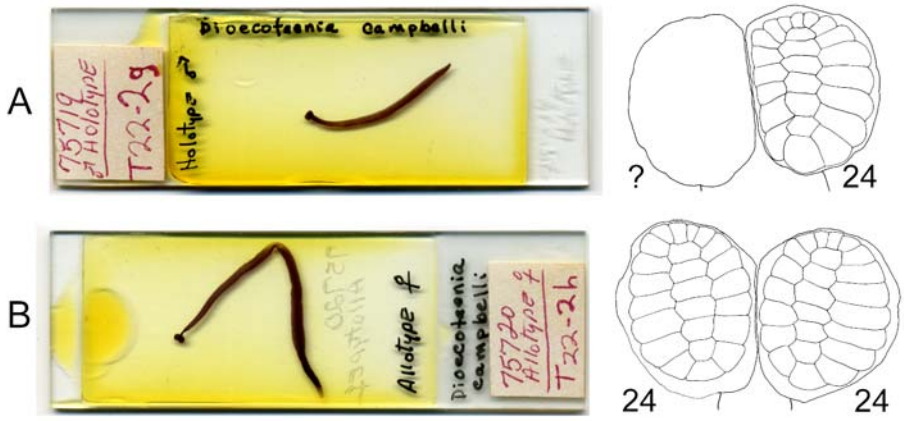

Figure 5. Type slides and line drawings of the corresponding scolices of *Dioecotaenia campbelli* indicating the number of bothridial loculi. (A) Holotype (USNPC No. 75719). (B) Allotype (USNPC No. 75720).

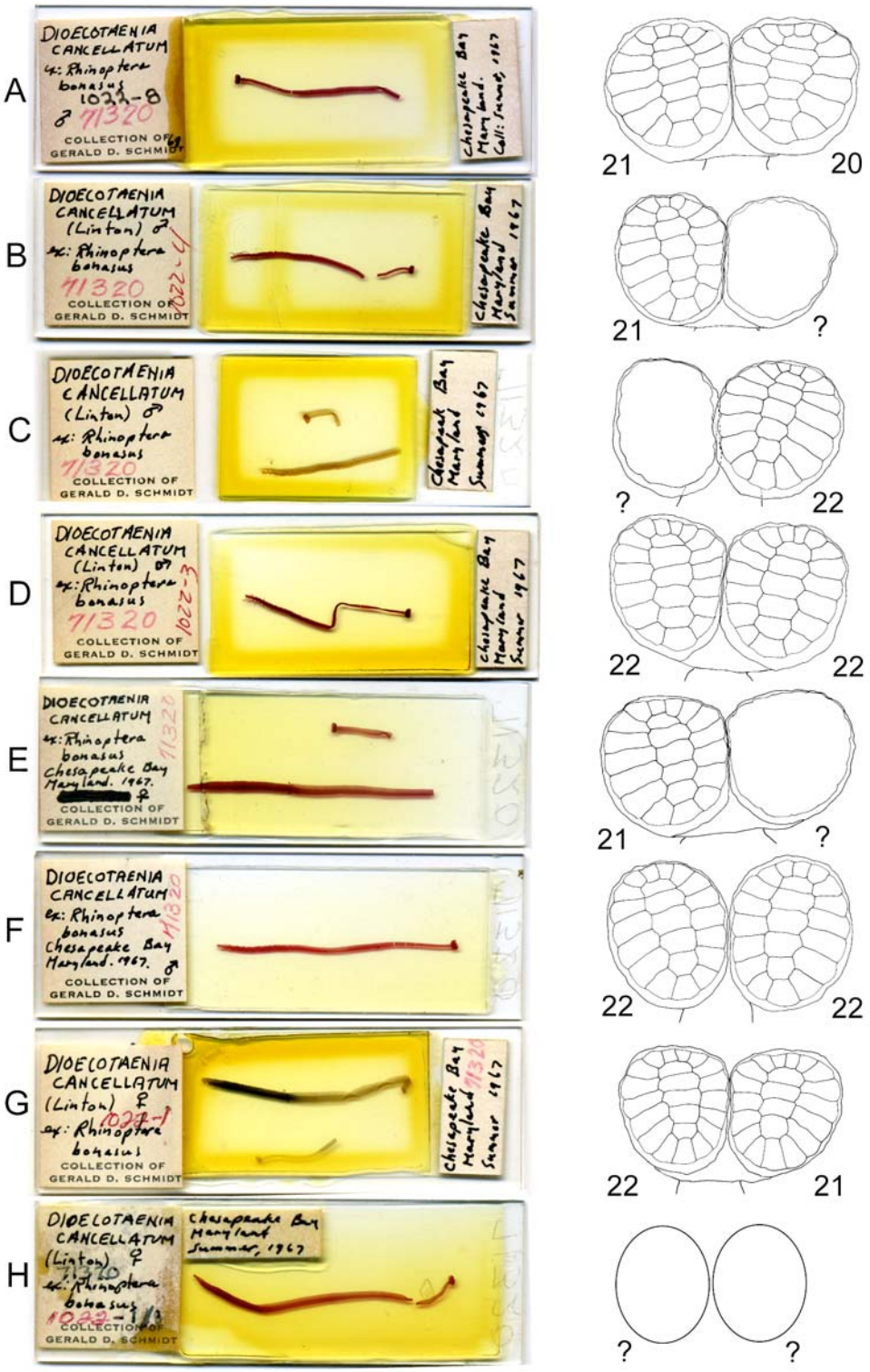

Figure 6. Voucher slides and line drawings of the corresponding scolices of *Dioecotaenia cancellata* indicating the number of bothridial loculi. (A-H) Eight vouchers (USNPC No. 71320).

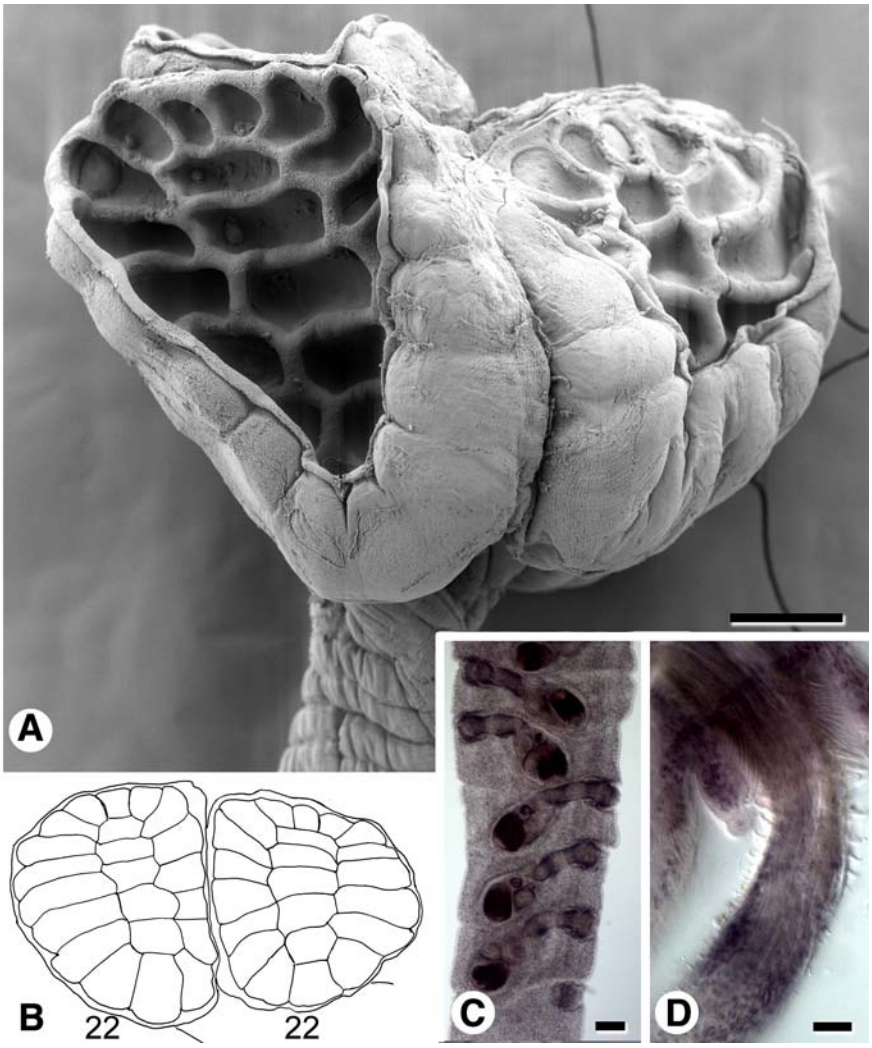

Figure 7. Scanning electron micrograph, line drawing, and light micrographs of *Dioecotaenia cancellata*. (A) Scolex. (B) Scolex indicating number of bothridial loculi. (C) Male proglottids. (D) Everted cirrus. Scale bars: A, C, 100 µm; D, 20 µm.

Rhodobothrium paucitesticulare Mayes and Brooks, 1981

(Fig. 8)

Material examined: USNPC No. 75717 (holotype); USNPC No. 75718 (paratype).

Voucher specimens deposited: Two whole mounted vouchers (USNPC No. 00000).

The possession of four pedicellated bothridia with the adherent surface forming numerous convolutions, as well as proglottid morphology with testes in immature segments that degenerated as the segments mature identify specimens collected in this study as *R. paucitesticulare*.

Campbell and Carvajal (1979) recognized four additional species, all in the genus *Rhodobothrium* Linton, 1889, from dasyatid and myliobatid rays: *Rhodobothrium pulvinatum* Linton, 1889; *R. lubeti* (Euzet, 1959) Campbell and Carvajal, 1979; *R. mesodesmatum* (Bahamonde and Lopez, 1962) Campbell and Carvajal, 1979; and *R. brachyascum* (Riser, 1955) Campbell and Carvajal, 1979.

Two paratypes and specimens collected as part of this study possess convolutions of the bothridia, which are much more evident than illustrated in the original description. The newly collected specimens possessed 40-65 (49 ± 8; 15; 5) testes, well within the range given by Mayes and Brooks (1981) of 40-80 (50). The number of testes is the feature that distinguishes *R. paucitesticulare* from all other species in the genus with *R. pulvinatum, R. lubeti,* and *R. mesodesmatum*, which possess 117-149, 120-160, and 150- 210 testes, respectively. Campbell and Carvajal (1979) considered *R. brachyascum* to be of questionable generic status due to the

bothridia being thin and leaf-like, and more like those of members of the genus *Anthobothrium* Van Beneden, 1850. The bothridia of the specimens from this study are neither thin nor leaf-like. No further distinguishing feature for *R. brachyascum* was given.

Information on the microthrix pattern of *R. paucitesticulare* was not presented by Mayes and Brooks (1981). Scanning electron microscopy conducted as part of this study shows short filitriches covering the distal bothridial surface (Fig. 8 B), rim of the bothridia (Fig. 8 C), the proximal bothridial surface, and scolex proper.

Rhodobothrium paucitesticulare was present in six of the 12 cownose rays.

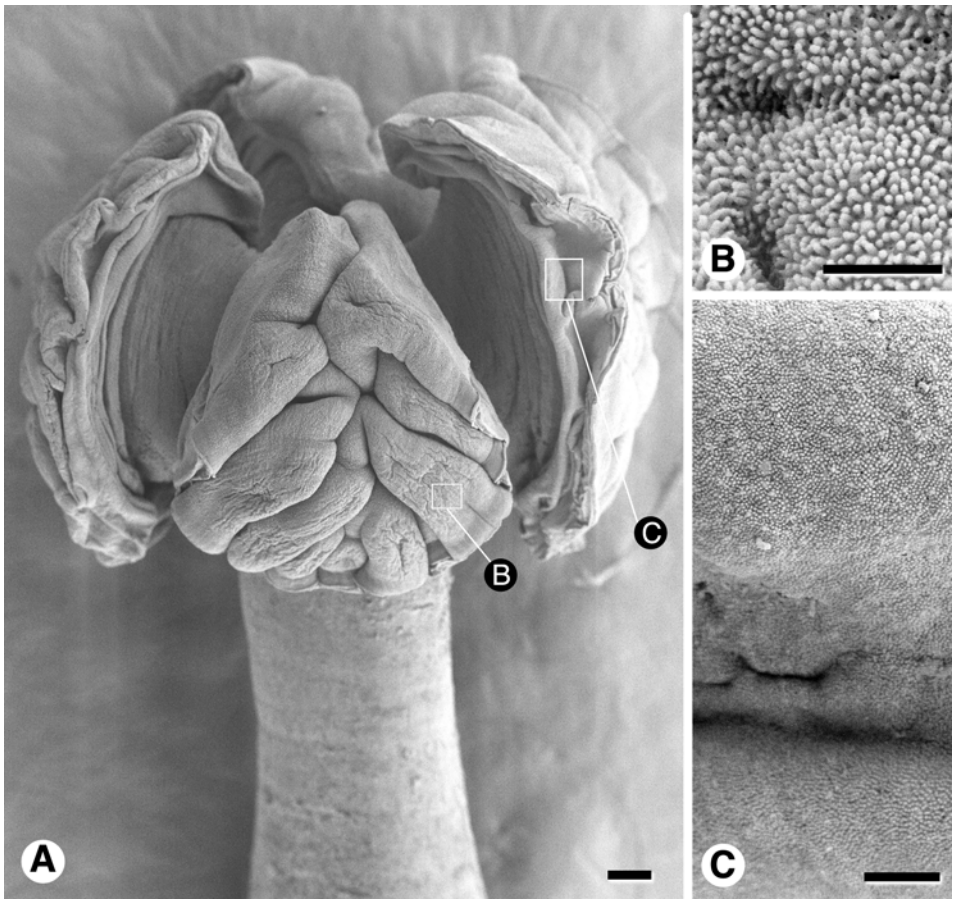

Figure 8. Scanning electron micrographs of *Rhodobothrium paucitesticulare*. (A) Scolex; boxes indicate where Fig. 8B-C were taken. (B) Distal bothridial surface. (C) Rim of bothridium. Scale bars: A, 100 µm; B, 1 µm; C, 2 µm.

Tylocephalum bonasum Williams and Campbell, 1984

(Fig. 9)

Voucher specimens deposited: One whole mounted voucher (USNPC No. 00000).

Only five specimens identified as belonging to *Tylocephalum* Linton, 1890, were collected in this study, only one of these was mature. Of the three species of *Tylocephalum* known to parasitize *R. bonasus* (i.e., *T. pingue, T. brooksi,* and *T. bonasum*), the specimens collected as part of this study possessed a scolex most similar to that of *T. bonasum*. Although the apical organ (i.e., metoporhynchus) is disrupted, it is more protruded than in *T. pingue*. In addition, the genital pore is located slightly more anteriorly in the proglottid in *T. bonasum* as opposed to medially in *T. pingue*. The single collected specimen possessing mature proglottids of *T. bonasum* differs from *T. brooksi* by lacking postovarian vitellaria and a large external seminal vesicle.

Jensen (2005) recognized as valid an additional nine species in the genus *Tylocephalum* from a diversity of hosts (dasyatids, rhinobatids, a squatinid, and a myliobatid): *Tylocephalum campanulatum* Butler, 1987, *T. elongatum* Subhapradha, 1955, *T. marsupium* Linton 1916, *T. pandurangi* Shinde and Mahajan, 1995, *T. rhinobatii* (Deshmukh, 1980), *T. singhii* Jadhav and Shinde, 1981, *T. squatinae* Yamaguti, 1934, *T. yorkei* Southwell, 1925, and *T. koenneckeorum* Jensen, 2005. The collected specimen of *T. bonasum* differs from *T. campulatum, T. elongatum, T. rhinobatii, T. squatinae* and *T. yorkei* in possessing a greater number of testes per

proglottid (72-80 [68-93 in original description] vs. 40-50, 40, 27-30, 40-50, and 26-30, respectively), and fewer testes than *T. pandurangi* (110-120). Furthermore, the single mature specimen of *T. bonasum* possessed 331 proglottids, which distinguishes it from *T. singhii* and *T. koenneckeorum*, in which the maximum number of proglottids is 20 and 80, respectively. The present specimen of *T. bonasum* lacks an expanded seminal vesicle, which distinguishes it from *T. marsupium,* which possesses a voluminous seminal vesicle.

The surface ultrastructure of *T. bonasum* has not previously been presented. Due to the disruption of the surface of the apical organ, it is possible only to describe microtriches on the scolex proper. The surface of the scolex proper is covered with long filitriches (Fig. 9 A). This pattern is consistent with the microthrix pattern described for *T. brooksi* by Ivanov and Campbell (2000) and *T. koenneckeorum* by Jensen (2005).

Tylocephalum bonasum was present in two of the 12 cownose rays.

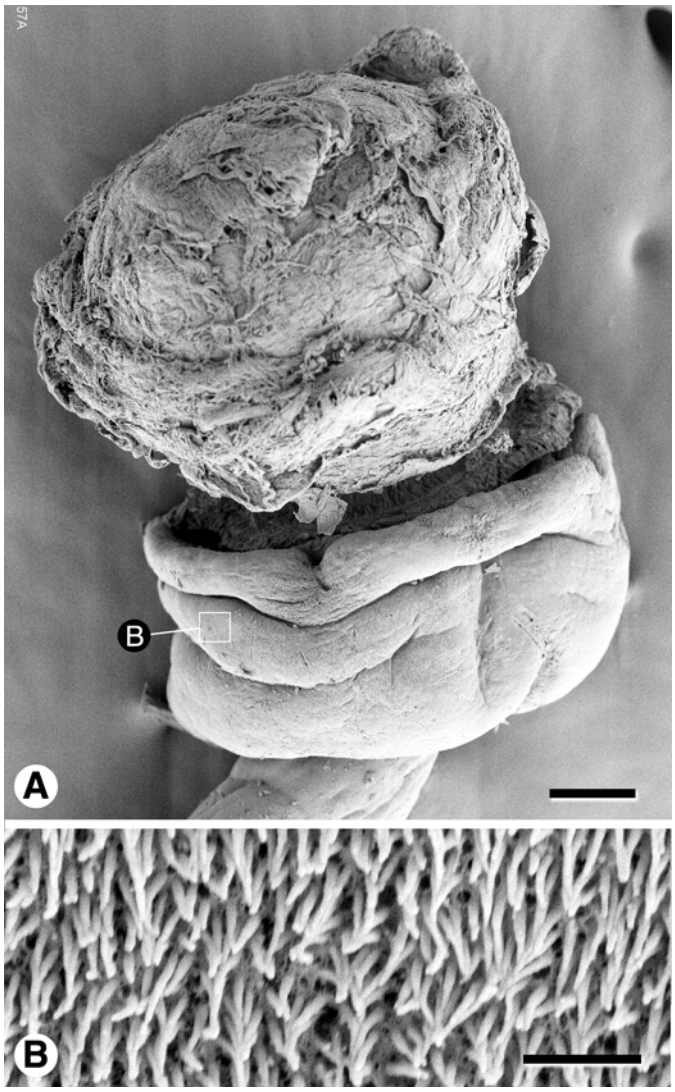

Figure 9. Scanning electron micrographs of *Tylocephalum bonasum*. (A) Scolex; box indicates where Fig. 9B was taken. (B) Scolex proper surface. Scale bars: A, 100 µm; B, 1 µm.

Redescribed Species

Duplicibothrium minutum (Williams and Campbell, 1978)

(Figs. 10, 11)

Description

Based on the holotype (USNPC No. 74724), four paratypes (USNPC Nos. 74725, 74726; HWML No. 20884), 13 whole mounted voucher specimens, and two specimens prepared for SEM.

Worms 2,847-8,808 (5,011 ± 1,588.3; 16) long. Maximum width 193-407 (310 ± 53.5; 16) at level of scolex, immature proglottid, or mature proglottid. Proglottids 7-26 (16 ± 5; 16) in number, craspedote, euapolytic. Scolex 221-430 (322 ± 59.3; 16) long by 193-407 (276 ± 51.8; 16) wide bearing four bothridial acetabula. Acetabula fused in back-to-back pairs, each acetabulum 187-379 (296 ± 56.5; 16; 30) long by 99-182 (149 ± 20.6; 16; 30) wide, consisting of (from anterior to posterior) single apical loculus, 2 lateral loculi, 7-8 rows with a single loculus, 1 row of 3 loculi, and a most posterior row of 7 loculi (Fig. 10 B).

Distal bothridial surface of apical loculus (Fig. 11 B), posterior loculi (Figs. 11 C), proximal bothridial surface, scolex proper and cephalic peduncle covered with short filitriches. Cephalic peduncle present, 864-4,224 (2,058 ± 808.2; 16) long by 64-210 (140 ± 33.4; 16) wide.

Immature proglottids 6-25 (14 ± 5; 16) in number, gradually becoming longer than wide, two most posterior immature proglottids 151-542 (361 ± 99.9; 15; 30) long by 157-382 (291 ± 68.1; 15; 30) wide. Mature proglottids 1-2 in number, longer than

wide; terminal proglottid 506-1,023 (750 ± 161.7; 16) long by 125-344 (274 ± 54.3; 16) wide. Gravid proglottids not observed.

Testes 24-31 (26 ± 2; 16) in number, degenerating in more mature proglottids, 29-99 (55 ± 15.4; 15; 45) long by 35-129 (79 ± 16.4; 15; 45) wide, distributed in two columns, extending entire length of proglottid uninterrupted by ovary, poral column interrupted by cirrus sac. Cirrus sac pyriform, 85-184 (123 ± 23.9; 14) long by 42-109 (73 ± 19.4; 14) wide, containing armed cirrus. Vas deferens weakly developed, extending from level of ootype to level of genital pore, becoming voluminous and more convoluted in more mature proglottids. Ovary digitiform in dorsoventral view (Fig. 10 C), radiating from central isthmus, 191-400 (281 ± 61.5; 14) long by 151-264 (201 ± 35.1; 14) wide. Vagina thin-walled, medial, extending from genital pore to ootype. Genital pores submarginal, irregularly alternating, 79-93% (86 ± 4.7; 14) of proglottid length from posterior end. Uterus median, extending from ootype to anterior margin of cirrus sac; uterine duct not observed. Vitellaria follicular, circumcortical, 10-43 (19 ± 6.4; 15; 45) long by 7-48 (26 ± 10.3; 15; 45) wide. Dorsal and ventral excretory ducts observed.

Taxonomic Summary

Type Host: *Rhinoptera bonasus* (Mitchill), Cownose ray (Rhinopteridae, Myliobatiformes).

Additional Hosts: None.

Site of Infection: Spiral Intestine.

Type Locality: Chesapeake Bay, Virginia, U.S.A.

Additional Localities: Sakonnet Point, Rhode Island, U.S.A. (Williams & Campbell, 1978); Ship Island, Mississippi, Gulf of Mexico, U.S.A. (this study); Horn Island, Mississippi, Gulf of Mexico, U.S.A. (this study); Chandeleur Islands, Louisiana, Gulf of Mexico, U.S.A. (this study).

Type specimens: Holotype (USNPC No. 74724), four paratypes (USNPC Nos. 74725, 74726, HWML No. 20884),

Voucher specimens deposited: Five (USNPC Nos. 00000), three (LRP Nos. 00000), and five whole mounted vouchers (KUNHM Nos. 00000)

Etymology: This species was named for its relatively small size (*minutum*, L, small).

Remarks

The possession of bothridial acetabula fused along their length in back-to-back pairs, bothridial surfaces divided into loculi by horizontal and longitudinal septa, a submarginal genital pore, and a digitiform ovary clearly place this in *Duplicibothrium*.

The description presented here is based on measurements of five of the seven type specimens and measurements of additional specimens collected from the northern Gulf of Mexico. The scolex of the species is reinterpreted. Most measurements overlap or expand the ranges given in the original description. Measurements of features, not included in the original description, were taken.

Examination of the type material revealed a discrepancy between the interpretation of the scolex presented in the original description and the features observed in the type material. In the original description, *D. minutum* was said to possess six to eight horizontal loculi and the posterior region of the bothridia were described as superficially subdivided. The original illustrations depict six to seven posterior loculi. Upon examination of the type material and newly collected specimens, the arrangement of bothridial loculi were interpreted as consisting of (from anterior to posterior) an apical loculus, two lateral loculi, seven to eight rows with a single loculus, a horizontal row of three loculi, and a most posterior row of seven loculi (Figs. 10A, 12A).

The ranges of most measurements were consistent with those of the original description except for two: immature proglottid length (64-120 vs. 151-542) and cirrus sac length (47-83 vs. 85-184). The reason for this discrepancy may be due to the interpretation of Williams and Campbell (1978) that *D. minutum* possessed more mature proglottids than interpreted in this description. Therefore, Williams and Campbell (1978) measured smaller, more anterior, immature proglottids. Alternatively, the most posterior immature proglottids might not have been the ones measured by Williams and Campbell (1978). The discrepancy in cirrus sac length can also be explained by this difference in interpretation of maturity, assuming that smaller cirrus sacs in immature proglottids were measured for the original description.

New measurements and counts include number of immature and mature proglottids, genital pore position, ovary length and width, and the position of the ovarian bridge.

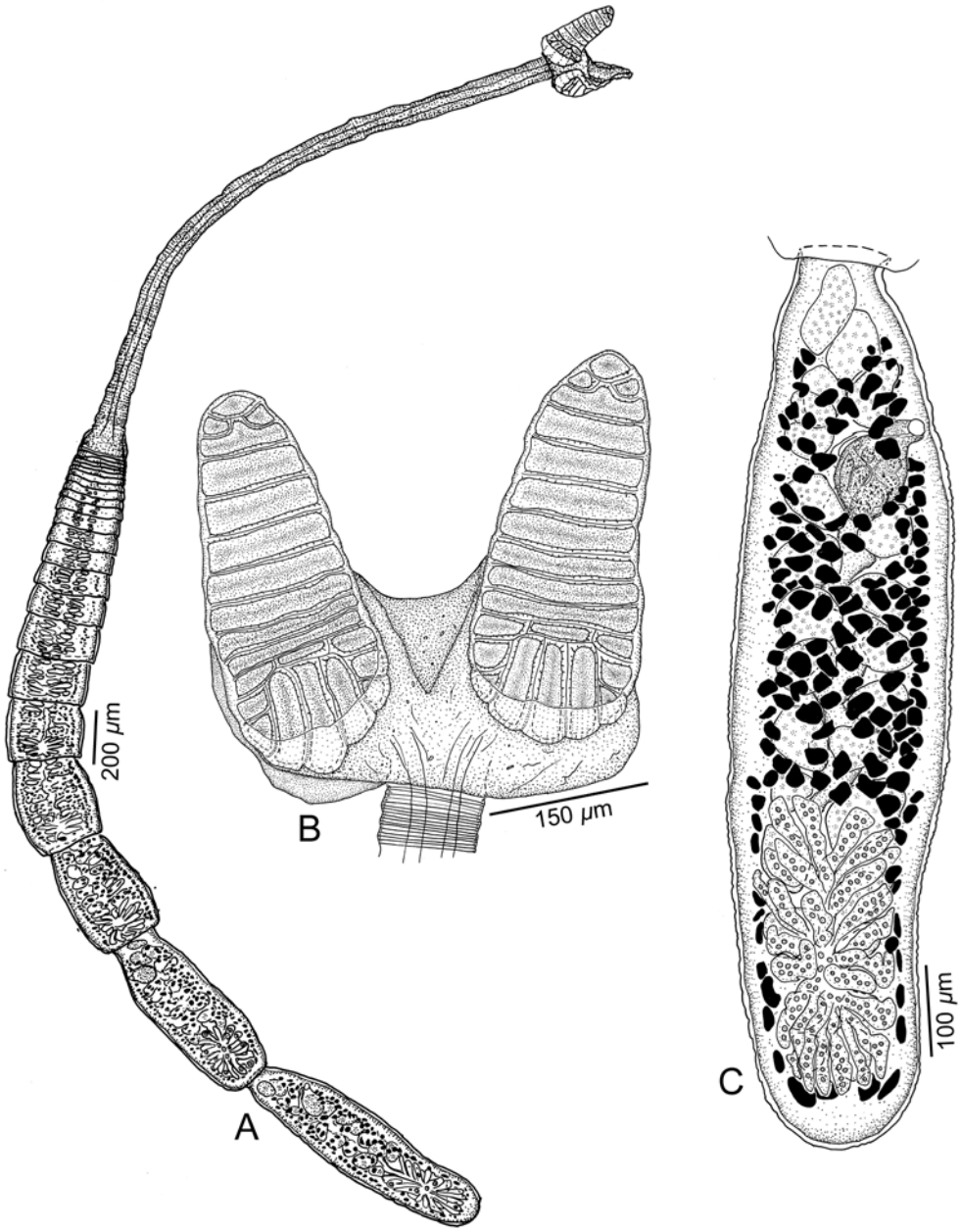

Figure 10. Line drawings of *Duplicibothrium minutum*. (A) Whole worm (USNPC No. 00000). (B) Scolex (USNPC No. 00000). (C) Mature proglottid (USNPC No. 00000).

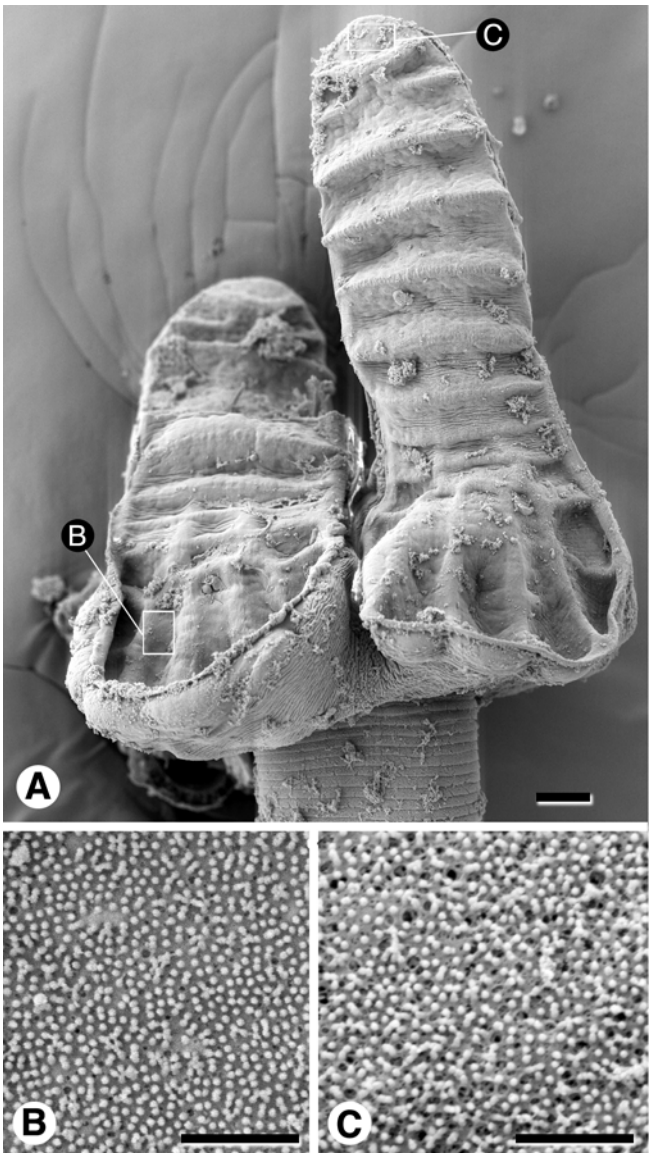

Figure 11. Scanning electron micrographs of *Duplicibothrium minutum*. (A) Scolex; boxes indicate where Fig. 11B-C were taken. (B) Distal bothridial surface of posterior loculus. (C) Distal bothridial surface of anterior loculus. Scale bars: A, 20 µm; B-C, 1 µm.

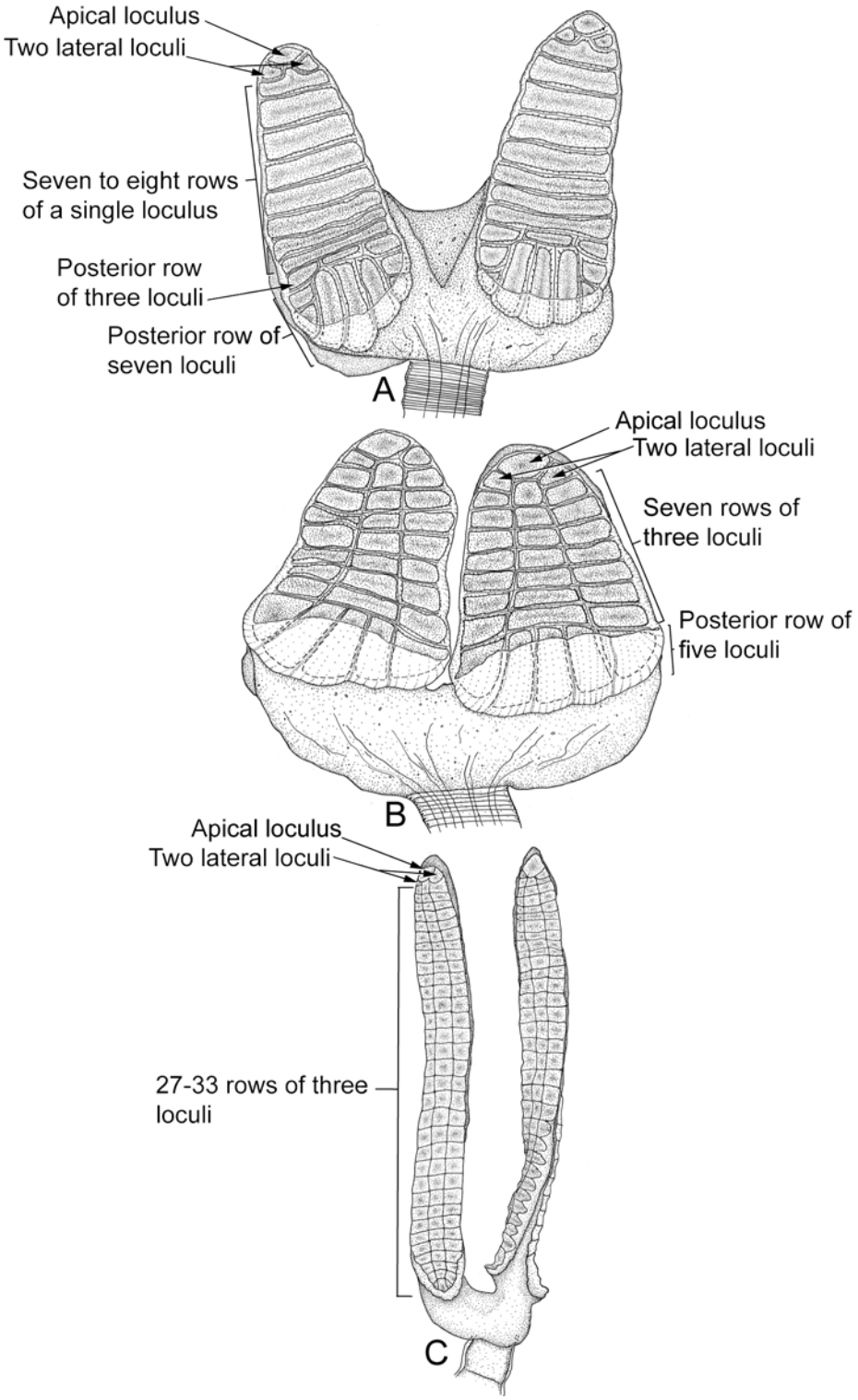

Figure 12. Interpretation of loculi counts of *Duplicibothrium* species used in this study. (A) *D. minutum*. (B) *D. karenae* n. sp. (C) *D. mergacephalum* n. sp.

Descriptions of New Species

Duplicibothrium karenae n. sp.

(Figs. 13, 14)

Description

Based on 16 whole mounts and one specimen prepared for SEM.

Worms 7,372-16,253 (10,703 ± 2,409.3; 16) long. Maximum width 475-792 (635 ± 98; 14) at level of scolex. Proglottids 23-41 (31 ± 6; 15) in number, craspedote, euapolytic. Scolex 430-616 (526 ± 61.7; 11) long by 475-792 (635 ± 98; 14) wide, bearing four bothridial acetabula. Acetabula fused in back-to-back pairs, each acetabulum 375-531 (454 ± 46.6; 9; 17) long by 260-432 (341 ± 45.9; 11; 22) wide, with (from anterior to posterior) single apical loculus, 2 lateral loculi, 7 rows of 3 loculi, and a posterior row with 5 loculi (Fig. 13 A). Distal bothridial surface (Fig. 14 B), proximal bothridial surface (Fig. 14C), scolex proper, and cephalic peduncle covered with short filitriches. Cephalic peduncle present, 1,179-5,699 (3,273 ± 1,246; 16) long by 113-233 (179 ± 31.0; 16) wide.

Immature proglottids 21-37 (27 ± 5; 15) in number, gradually becoming longer than wide, two most posterior immature proglottids 321-679 (483 ± 107.0; 16; 32) long by 282-502 (349 ± 51.3; 16; 32) wide. Mature proglottids 2-5 (3.4 ± 1; 16) in number, longer than wide; terminal proglottid 957-1,440 (1,171 ± 161.5; 15) long by 283-376 (336 ± 33.8; 15) wide. Gravid proglottids not observed. Testes 32-50 (39 ± 5; 16) in number, degenerating in more mature proglottids, 36-110 (62 ± 13; 16; 48) long by 56-123 (87 ± 16.9; 16; 48) wide, distributed in two columns, extending entire

length of proglottid uninterrupted by ovary, poral column interrupted by cirrus sac. Cirrus sac pyriform, 131-185 (163 ± 15.6; 16) long by 55-123 (91 ± 15.5; 16) wide, containing armed cirrus. Vas deferens extending from level of ootype to level of genital pore, becoming voluminous and more convoluted in more mature proglottids. Ovary digitiform in dorsoventral view (Fig. 13 C), radiating from central isthmus, 219-510 (387 ± 75.0; 15) long by 166-280 (224 ± 35.9; 15) wide. Vagina thin-walled, medial, extending from genital pore to ootype. Genital pores submarginal, irregularly alternating, 82-90% (85 ± 1.9; 16) of proglottid length from posterior end. Uterus median, extending from ootype to anterior margin of cirrus sac; uterine duct not observed. Vitellaria follicular, circumcortical, 8-43 (21.8 ± 8.2; 16; 48) long by 17-44 (31 ± 7.3; 16; 48) wide. Dorsal and ventral excretory ducts observed.

Taxonomic Summary

Type host: *Rhinoptera bonasus* (Mitchill), Cownose ray (Rhinopteridae, Myliobatiformes).

Additional Hosts: None.

Site of Infection: Spiral Intestine.

Type Locality: Horn Island, Mississippi, Gulf of Mexico, U.S.A.

Additional Localities: Ship Island, Mississippi, Gulf of Mexico, U.S.A.

Type Specimens: Holotype (USNPC No. 00000), five paratypes (USNPC Nos. 00000); five paratypes (LRP Nos. 00000); five paratypes (KUNHM Nos. 00000).

Etymology: This species is named in honor of the author's mother, Karen Call, who has given unconditional support and encouragement to all of the author's endeavors.

Remarks

Bothridial acetabula fused lengthwise in back-to-back pairs, bothridial surfaces divided into loculi by horizontal and longitudinal septa, submarginal genital pore, and a digitiform ovary clearly place this species in the genus *Duplicibothrium*.

Currently three species are recognized in the genus *Duplicibothrium* (Ruhnke et al., 2000): *Duplicibothrium cairae*; *D. paulum;* and *D. minutum*. *Duplicibothrium karenae* differs from *D. cairae, D. minutum*, and *D. paulum* in the number of loculi in the most posterior row (5 vs. 7, 7, 3). In addition, *D. karenae* possesses a longer terminal proglottid than *D. cairae* (957-1,440 vs. 355-893). *Duplicibothrium karenae* is further distinguished from *D. minutum* in that *D. karenae* possesses wider bothridia (260-432 vs. 99-182) and seven rows of three loculi versus one row of three loculi (Fig. 12 A, B). *Duplicibothrium karenae* can be further distinguished from *D. paulum* based on its greater length (7,372-16,253 vs. 700-2,900), and possession of a cephalic peduncle, which is lacking in *D. paulum*.

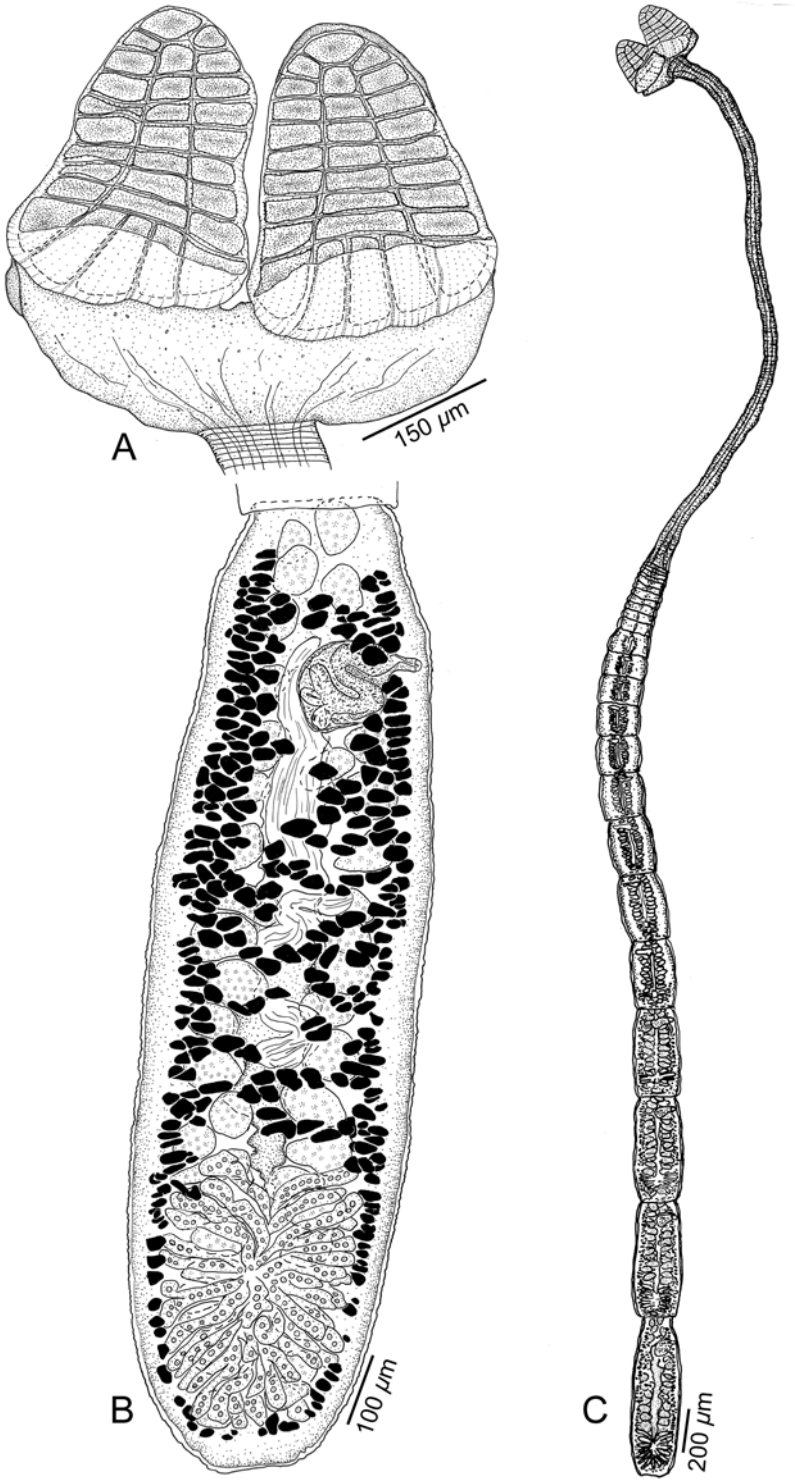

Figure 13. Line drawings of *Duplicibothrium karenae* n. sp. (A) Scolex (USNPC No. 00000). (B) Mature proglottid (USNPC No. 00000). (C) Whole worm (USNPC No. 00000).

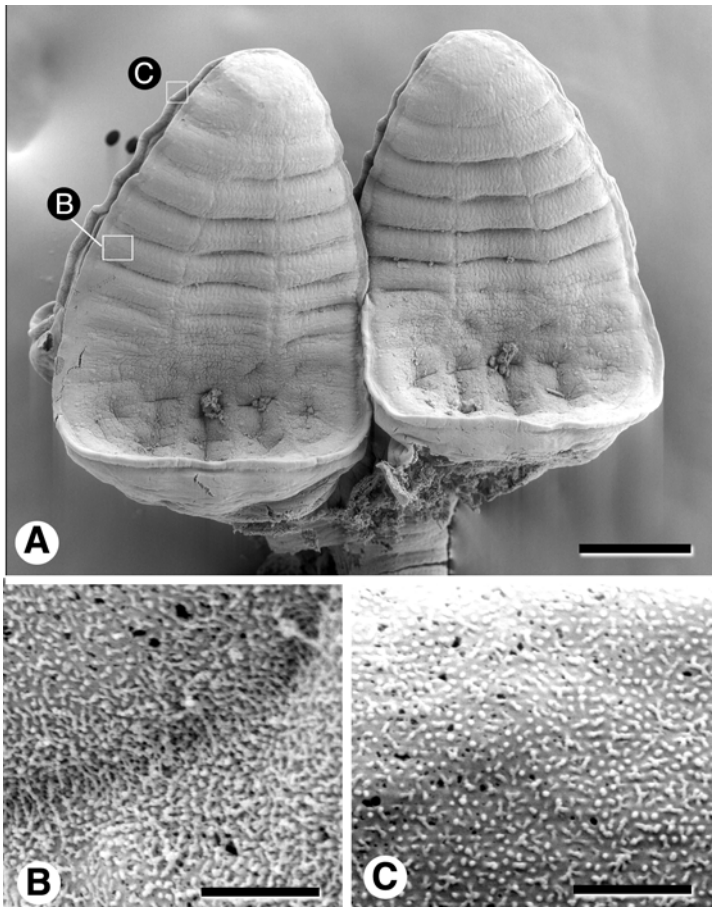

Figure 14. Scanning electron micrographs of *Duplicibothrium karenae* n. sp. (A) Scolex; boxes indicate where Fig. 14B-C were taken. (B) Distal bothridial surface. (C) Proximal bothridial surface. Scale bars: A, 100 µm; B-C, 1 µm.

Duplicibothrium mergacephalum n. sp.

(Figs. 15, 16)

Description

Based on 17 whole mounts, one specimen cross-section series, and two specimens prepared for SEM.

Worms 1,338-2,210 (1,717 ± 253.7; 16) long. Maximum width 221-324 (259 ± 26.7; 16) at terminal proglottid or scolex. Proglottids 5-6 (5 ± 1; 16) in number, craspedote, euapolytic. Scolex 613-946 (793 ± 104.7; 16) long by 210-324 (247 ± 94.5; 16), bearing four bothridial acetabula. Acetabula fused in back-to-back pairs, each acetabulum 533-887 (710 ± 94.5; 16; 30) long by 63-98 (79 ± 10.4; 15) wide, with (from anterior to posterior) single apical loculus, 2 lateral loculi, and 27-33 rows of 3 loculi (Fig. 15A). Distal bothridial surface (Fig. 16 B), proximal bothridial surface, and scolex proper (Fig. 16 C) covered with short, round filitriches. Reproductive proglottids covered with long filitriches (Fig. 16 D). Cephalic peduncle absent.

Immature proglottids 4-5 (4 ± 1; 16) in number, gradually becoming longer than wide, two most posterior immature proglottids 57-311 (158 ± 74.1; 16; 32) long by 120-240 (167 ± 28.7; 16; 32) wide. Mature proglottids 1 in number, longer than wide, 330-810 (555 ± 132.9; 16) long by 206-281 (238 ± 22.6; 16) wide. Gravid proglottids not observed. Testes 18 in number, 1-2 rows deep in cross-section (Figs. 15 D, E), degenerating in more mature proglottid, 28-72 (49 ± 12.4; 14; 41) long by 36-77 (51 ± 9.6; 14; 41) wide, distributed in two columns, extending entire length of

proglottid interrupted by ovary, poral column interrupted by cirrus sac, post-ovarian testes present. Cirrus sac pyriform, 95-163 (134 ± 17.3; 16) long by 52-129 (85.9 ± 23.1; 16) wide, containing armed cirrus. Ovary digitiform in dorsoventral view (Fig. 15 C), radiating from central isthmus (Fig. 15 C, E), 104-301 (190 ± 50.6; 16) long by 64-188 (151 ± 32.4; 16) wide, digitiform in cross-section. Vagina convoluted, sinuous, thick-walled, medial, extending from genital pore to ootype. Genital pores submarginal, irregularly alternating, 73-85% (76 ± 3.1; 16) of proglottid length from posterior end. Uterus median, extending from ootype to anterior margin of cirrus sac; uterine duct not observed. Vitellaria follicular, circumcortical, 9-47 (23 ± 9.8; 16; 48) long by 10-42 (24 ± 7.4; 16; 48) wide. Dorsal and ventral excretory ducts observed.

Taxonomic Summary

Type host: *Rhinoptera bonasus* (Mitchill), Cownose ray (Rhinopteridae, Myliobatiformes)

Additional Hosts: None.

Site of Infection: Spiral Intestine.

Type Locality: Horn Island, Mississippi, Gulf of Mexico, U.S.A.

Additional Localities: None.

Type Specimens: Holotype (USNPC No. 00000), eight paratypes (seven whole mounts and one cross-section series) (USNPC Nos. 00000); five paratypes (LRP Nos. 00000); six paratypes (KUNHM Nos. 00000).

Etymology: *Merga (*L., two-pronged pitchfork) refers to the resemblance of the scolex to a pitch or tuning fork; *kephale* (Gr., head).

Remarks

Bothridial acetabula fused lengthwise in back-to-back pairs, bothridial surfaces divided into loculi by horizontal and longitudinal septa, submarginal genital pore, and a digitiform ovary clearly place this in the genus *Duplicibothrium.*

Duplicibothrium mergacephalum can be distinguished from the other four known species, *D. cairae*, *D. karenae, D. minutum,* and *D. paulum,* in that it possesses a greater number of horizontal rows of loculi (27-33 vs. 8-9, 8, 9-10, and 18-20 respectively) (Fig. 12 A-C). In addition, *D. mergacephalum* is distinguished from *D. cairae, D. karenae,* and *D. minutum* in that it possesses fewer loculi in the most posterior row (3 vs. 7, 5, 7, respectively) (Fig. 12 A-C), lacks a cephalic peduncle, and possesses fewer testes (18 vs. 28-43, 32-50, 24-31, respectively). *Duplicibothrium mergacephalum* also differs from *D. paulum* in the possession of narrower bothridia (63-98 vs. 120-336).

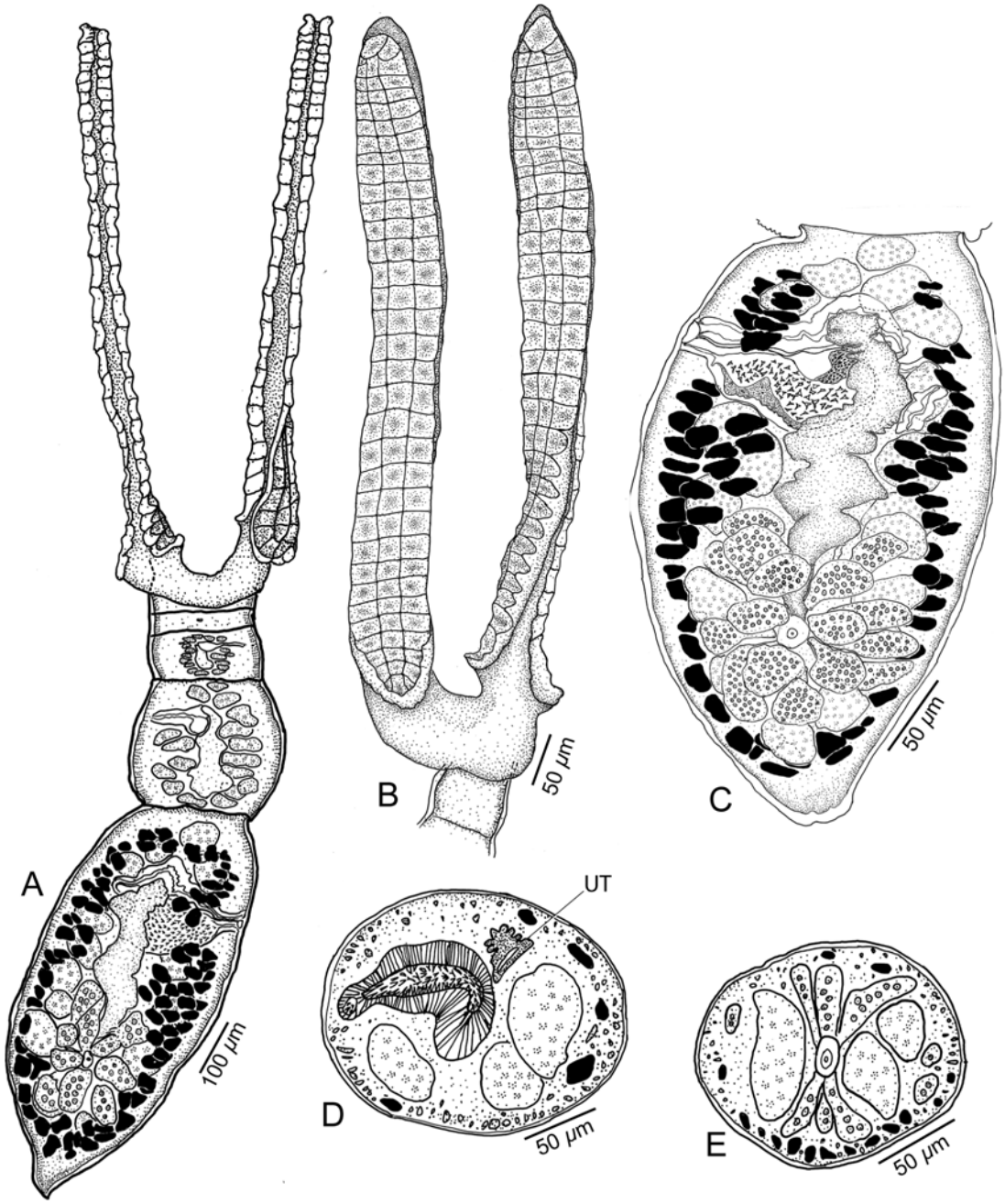

Figure 15. Line drawings of *Duplicibothrium mergacephalum* n. sp. (A) Whole worm (USNPC No. 00000). (B) Scolex (USNPC No. 00000). (C) Mature proglottid (USNPC No. 00000). (D) Cross-section at level of genital pore (USNPC 00000). (E) Cross-section at level of ovarian bridge (USNPC No. 00000).

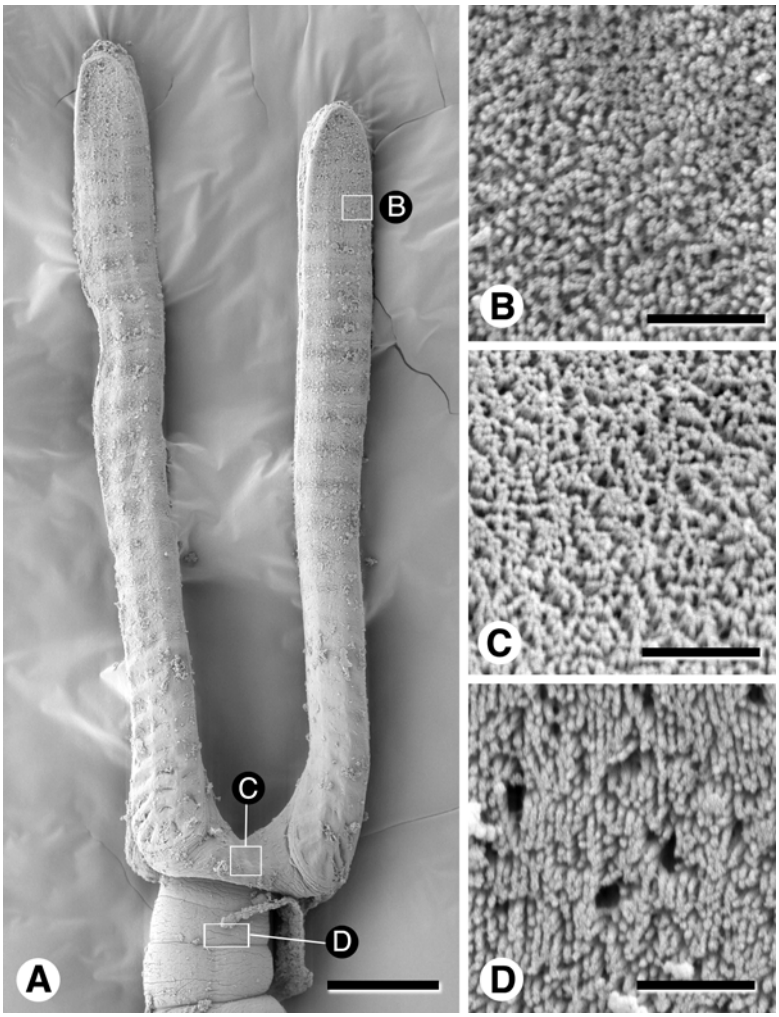

Figure 16. Scanning electron micrographs of *Duplicibothrium mergacephalum* n. sp. (A) Scolex; boxes indicate where Fig. 16B-D were taken. (B) Distal bothridial surface. (C) Scolex proper surface. (D) Strobila surface. Scale bars: A, 100 μm; B-D, 1 μm.

Eniochobothrium overstreeti n. sp.

(Figs. 17, 18)

Description

Based on 28 whole mounts (21 with scolices, seven without scolices), two specimen cross-section series, three lactophenol preparations of eggs, and two specimens prepared for SEM.

Worms with scolex 908-2,046 (1,314 ± 318.4; 21) long, those lacking a scolex 1,025-1,927 (1,399 ± 344; 7) long, maximum width at level of trough or terminal proglottid. Proglottids 23-39 (30 ± 4; 28) in number, craspedote, apolytic. Strobila divided into an anterior trough region and posterior reproductive region; trough region consisting of non-reproductive proglottids, expanded laterally, U-shaped in cross-section; reproductive region of strobila consisting of reproductive proglottids that mature and develop reproductive organs.

Scolex 75-119 (98 ± 12.6; 21) long by 64-105 (83 ± 10.4; 21) wide, bearing four acetabula and apical modification of scolex proper (Fig. 17 B). Acetabula in form of suckers, sessile, 31-46 (40 ± 4.4; 21; 40) long by 23-37 (30 ± 3.8; 21; 39) wide. Apical modification of scolex proper, non-protrusible, with small apical aperture, housing apical organ. Apical organ glandular, non-eversible, non-protrusible, 26-57 (40 ± 7.0) long by 22-38 (27.7 ± 3.3) wide. Distal surfaces, rims of acetabula, and region of scolex proper between and slightly anterior and posterior to acetabula covered with spathate spinitriches (blade-like, *sensu* Jensen, 2001) and pointed spinitriches (Fig. 18 D, E, F). Scolex proper anterior to acetabula and apical

modification of scolex proper sparsely covered with triangular microtriches (Fig. 18 C). Non-reproductive proglottids covered with scale-like triangular microtriches, gradually shorter in length toward posterior proglottid margin (Fig. 18 G). Anterior-most immature reproductive proglottids covered with small triangular filitriches at proglottid margin becoming long filitriches anteriorly on proglottid (Fig. 18 H). Reproductive proglottids covered with long filitriches (Fig. 18 I).

Trough region 214-715 (332 ± 111.6; 21) long by 131-358 (246 ± 59.3; 21) wide, consisting of 14-28 (19 ± 3; 21) non-reproductive proglottids, with a straight edge at posterior margin; posterior margin 100-207 (148 ± 27.3; 27) wide. Reproductive region 473-1,729 (980 ± 316.6; 28) long by 197-390 (255 ± 48.6; 28) wide, consisting of 7-17 (11 ± 2; 28) reproductive proglottids; immature proglottids 6-16 (9 ± 2; 28) in number, wider than long, gradually increasing in length, rarely becoming longer than wide; posterior-most immature proglottid 42-389 (161 ± 82.9; 28) long by 173-272 (210 ± 27.0; 28) wide; mature proglottids 0-1 in number, longer than wide, 313-1,052 (519 ± 173.9; 19) long by 196-390 (234 ± 43.0;19) wide; gravid proglottids 0-1 in number 654-1,056 (798 ± 104.6; 14) long by 202-368 (270 ± 43.0; 14) wide. Testes 21-35 (24 ± 3; 19) in number, 9-48 (22 ± 8.6; 19; 57) long by 10-51 (26 ± 9.2; 19; 57) wide in mature proglottids, 1-2 rows deep in cross-section, distributed anterior to ovary, in aporal and poral fields; aporal field extending from anterior margin of proglottid to anterior margin of vitelline field; poral field with post-poral testes only, extending from the posterior region of cirrus sac to anterior margin of vitelline field; both fields slightly overlapping anterior margin of vitelline

field. Vas deferens extending anteriorly along lateral margin from ootype toward posterior margin of cirrus sac then toward distal end of cirrus sac along median of proglottid, entering the cirrus sac at its distal end. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac U-shaped, thick-walled, 221-460 (317.0 ± 63.7; 19) long by 47-86 (60.5 ± 9.6; 19) wide in mature proglottids, containing uncoiled cirrus. Cirrus armed. Ovary H-shaped in dorsoventral view (Fig. 17 C), bilobed in cross-section (Fig. 17 E) 54-210 (135.1 ± 47.1; 19) long by 73-167 (115.5 ± 24.1; 19) wide, lobulated. Vagina absent. Genital pores lateral, regularly alternating, 71-87% (77 ± 3.7; 19) of proglottid length from posterior end. Uterus median, extending from posterior margin of ovary to approximately posterior margin of cirrus sac; uterine duct not observed; uterine pore absent. Vitellaria follicular, medullary, in two lateral fields, multiple vitelline follicles on each side in cross-section, extending from posterior margin of cirrus sac to level of ovarian bridge; vitelline fields 59-198 (119 ± 42.9; 19) long, vitelline follicles 7-29 (16 ± 6.2; 19; 53) long by 11-45 (23 ± 7.9; 19; 53) wide. One dorsal and one ventral pair of excretory ducts. Eggs grouped in cocoons; each cocoon containing 28-42 (34 ± 4; 3; 19) eggs, free cocoons 75-110 (91 ± 10.4; 3; 20) long by 49-89 (67 ± 9.4; 3; 20) wide. Eggs subspherical, thin-walled. Oncospheres 13-23 (16 ± 2.5; 3; 27) long by 10-17 (13 ± 2.1; 3; 27) wide.

Taxonomic Summary

Type host: *Rhinoptera bonasus* (Mitchill), Cownose ray (Rhinopteridae, Myliobatiformes).

Additional Hosts: None.

Site of Infection: Spiral Intestine.

Type Locality: Horn Island, Mississippi, Gulf of Mexico, U.S.A.

Additional Localities: Ship Island, Mississippi, Gulf of Mexico, U.S.A.

Type Specimens: Holotype (USNPC No. 00000), seven paratypes (six whole mounts one cross-section series) (USNPC Nos. 00000); eight paratypes (seven whole mounts and one cross-section series) (LRP Nos. 00000); seven paratypes (KUNHM Nos. 00000); seven paratypes (HWML Nos. 00000).

Etymology: This species is named in honor of Dr. Robin Overstreet who graciously allowed the use of his laboratory at the Gulf Coast Research Laboratory to collect *R. bonasus*, and process parasite specimens.

Remarks

The two distinct regions of the strobila, (an anterior region consisting of laterally expanded, non-reproductive proglottids, forming a trough, and a reproductive region with proglottid internal reproductive anatomy), as well as the absence of a vagina, clearly identify this species as belonging to the genus *Eniochobothrium*.

Jensen (2005) recognized three species in the genus *Eniochobothrium* as valid; *Eniochobothrium gracile* Shipley and Hornell, 1906; *E. qatarense* Al Kawari, Saoud and Wanas, 1994; and *E. euaxos* Jensen, 2005. *Eniochobothrium overstreeti* can be distinguished from *E. qatarense* and *E. gracile* based on its shorter total length (980-2,046 vs. 3,250-5,650 and 3,500-12,000, respectively). In addition, in *E. overstreeti* the maximum width is less than in *E. qatarense* (206-390 vs. 600-850) and the mature proglottids are shorter (313-1,052 vs. 1,370-2,030). *Eniochobothrium overstreeti* is not as easily distinguished further from *E. gracile* due to the lack of detail provided in the original description (Shipley and Hornell, 1906). *Eniochobothrium overstreeti* does appear to possess a fewer proglottids (29-39 vs. 42-44). Furthermore, Shipley and Hornell (1906) described and illustrated *E. gracile* as possessing a short neck consisting of three proglottids; *E. overstreeti* lacks this region. *Eniochobothrium overstreeti* can be distinguished from *E. euaxos* based on the extent of the vas deferens and testes distribution. In *E. overstreeti* the vas deferens extends from the ootype to the distal end of the cirrus sac, whereas in *E. euaxos* the vas deferens extends from the ootype beyond the distal end of the cirrus sac to almost the anterior margin of proglottid before looping posteriorly and entering the cirrus sac at its distal end. Unlike *E. euaxos*, *E. overstreeti* does not possess testes on the poral side anterior to the genital pore, whereas *E. euaxos* possesses testes on the poral side of the proglottid anterior to the genital pore.

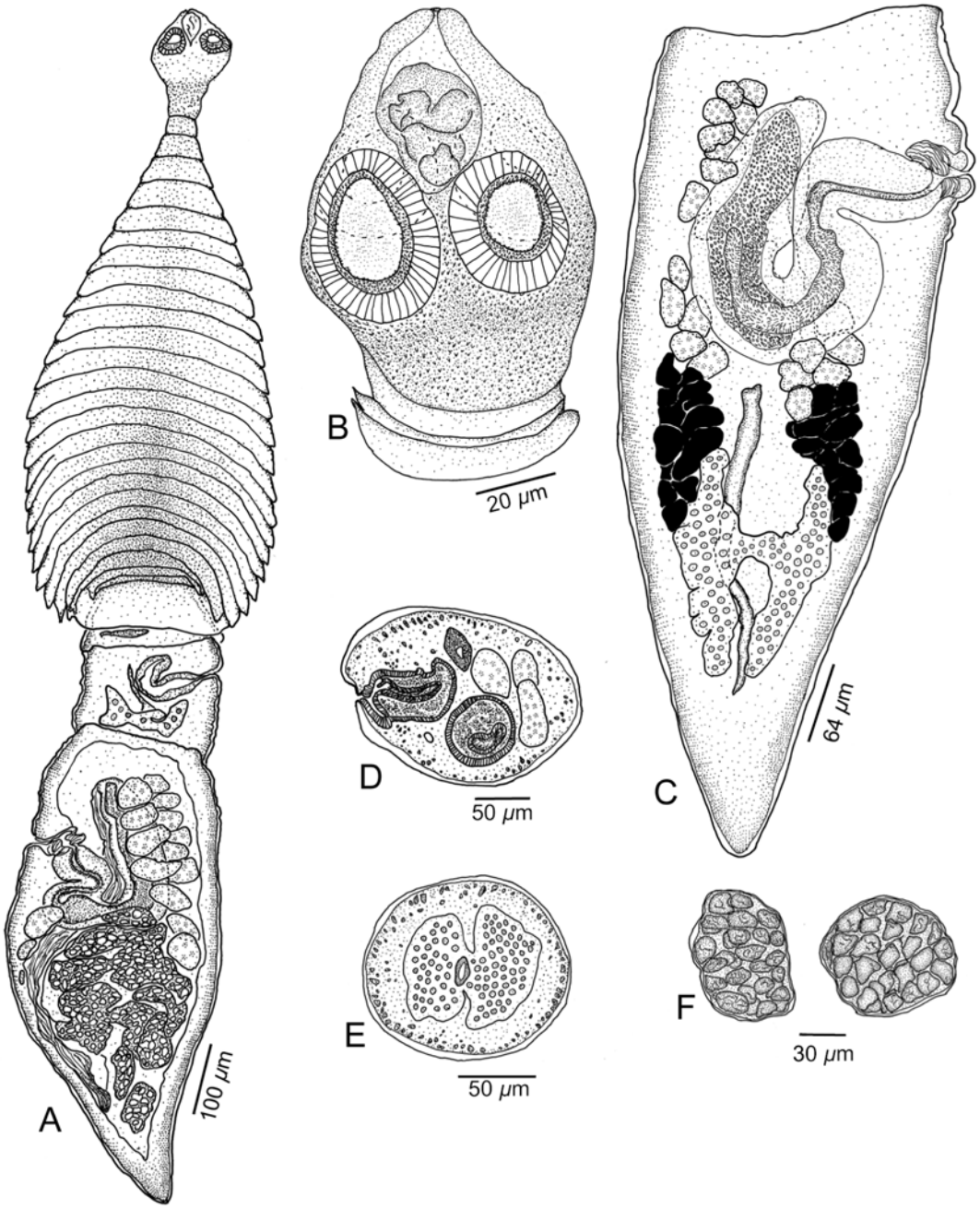

Figure 17. Line drawings of *Eniochobothrium overstreeti* n. sp. (A) Whole worm (USNPC No. 00000). (B) Scolex (USNPC No. 00000). (C) Mature proglottid (USNPC No. 00000). (D) Cross-section at level of genital pore (USNPC No. 00000). (E) Cross-section at level of ovarian bridge (USNPC No. 00000). (F) Cocoons.

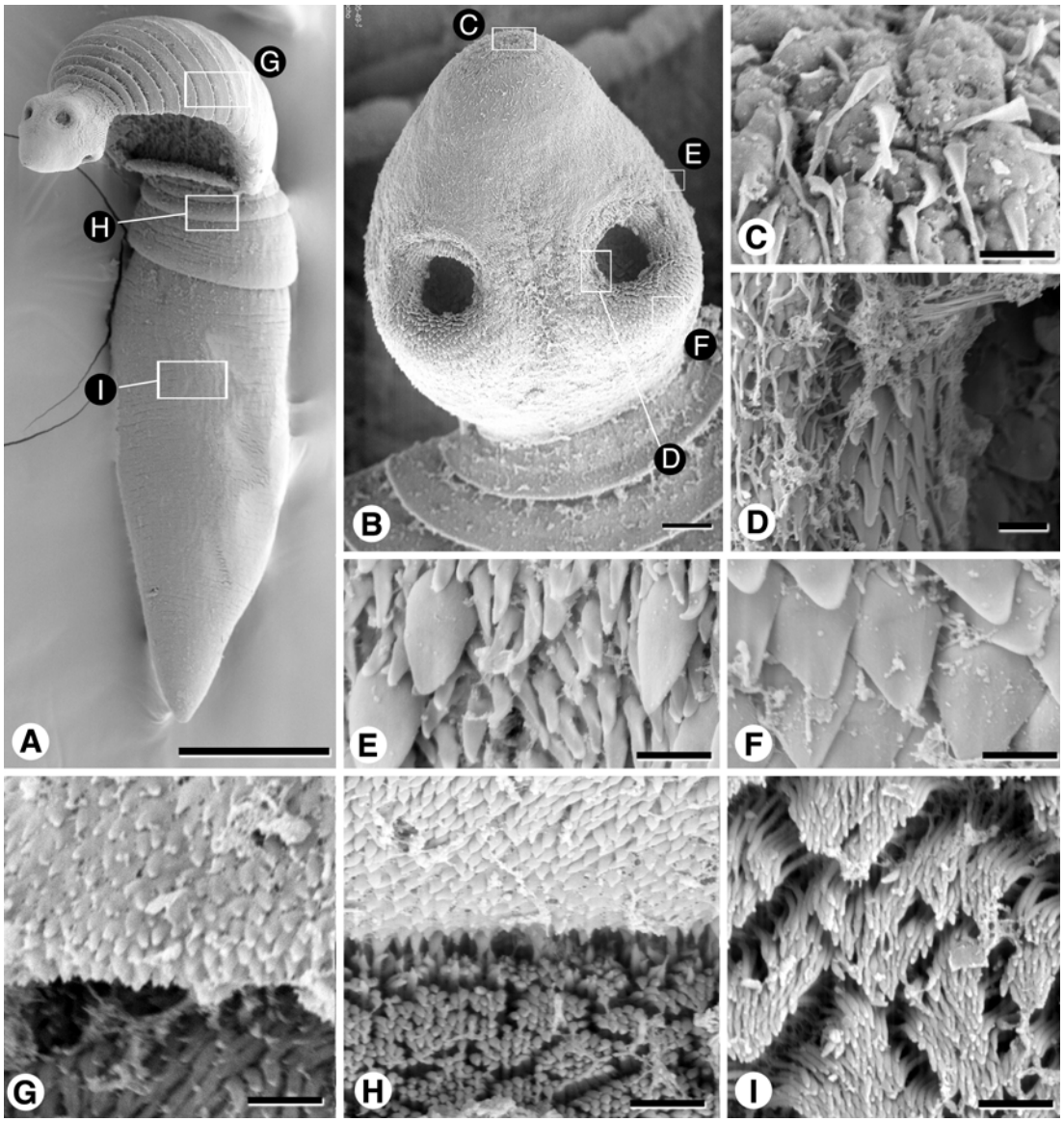

Figure 18. Scanning electron micrographs of *Eniochobothrium overstreeti*. (A) Whole worm; boxes indicate where Fig. 18G-I were taken. (B) Scolex; boxes indicate where Fig. 18C-F were taken. (C) Apical modification of scolex proper. (D) Rim of acetabula. (E) Surface between acetabula (F) Distal surface of acetabula. (G) Non-reproductive strobila surface. (H) Immature proglottid surface, near trough. (I) Reproductive strobila surface. Scale bars: A, 100 µm; B, 20 µm; C-I, 1 µm.

Eniochobothrium sedlockae n. sp.

(Figs. 19 A-J, 20 A-E)

Description

Based on 16 whole mounts (six with scolices, 10 without scolices), one specimen cross-section series, and two specimens prepared for SEM.

Worms with scolex 1,483-3,191 (2,211 ± 590.2; 6) long, those lacking a scolex 1,366-2,385 (1,842 ± 321.2) long, maximum width at level of trough or terminal proglottid. Proglottids, 30-45 (37 ± 5; 16) in number, apolytic. Strobila divided into an anterior trough region and posterior reproductive region; trough region consisting of non-reproductive proglottids U-shaped in cross-section; reproductive region of strobila consisting of reproductive proglottids which mature and develop reproductive organs.

Scolex 110-122 (117 ± 5.8; 4) long by 87-110 (98 ± 7.1; 6) wide, bearing four acetabula and apical modification of scolex proper (Fig. 19 A). Acetabula in form of suckers, sessile, 35-51 (45 ± 4.7; 6; 11) long by 27-40 (34 ± 4.8; 6; 12) wide. Apical modification of scolex proper, protrusible, with a small apical aperture housing apical organ. Apical organ glandular, non-eversible, non-protrusible, 38-51 (45 ± 5.5; 5) long by 29-34 (32 ± 2.5; 5) wide. Distal surfaces of acetabula covered with spathate (blade-like, *sensu* Jensen, 2001) spinitriches and long filitriches (Fig. 20 F). Rims of acetabula and scolex proper covered with small, triangular, pointed filitriches (Fig. 20 C, E). Apical modification of scolex proper covered with

wide triangular spinitriches and small, triangular, pointed filitriches (Fig. 20 D). Non-reproductive proglottids of trough covered with pointed scale-like microtriches that increase in length towards posterior proglottid margin (Fig. 20 G). Anterior-most immature reproductive proglottids covered with small triangular filitriches at proglottid margin becoming long filitriches anteriorly on proglottid (Fig. 20 H). Reproductive proglottids covered with long filitriches (Fig. 20 J).

Trough region 342-629 (495 ± 92.5; 6) long by 190-311 (243 ± 44.4; 5) wide, consisting of 19-23 (21 ± 2; 6) non-reproductive proglottids, with a rounded edge at posterior margin. Reproductive region 1,097-2,676 (1,570 ± 404.8; 15) long by 208-340 (284 ± 37.4; 15) wide, consisting of 14-25 (19 ± 3; 16) proglottids; immature proglottids 13-24 (18 ± 3; 16) in number, wider than long, gradually becoming longer than wide; posterior-most immature proglottid 82-401 (189 ± 90.3; 16) long by 125-285 (197 ± 42.8; 16) wide; mature proglottids 0-1 in number, longer than wide, 661-1,127 (922 ± 144.2; 12) long by 208-339 (275 ± 35.3; 112) wide; gravid proglottids 0-1 in number, 1,257-1,387 (1,322 ± 92.1; 2) long by 325-340 (333 ± 10.6; 2) wide; one spent proglottid observed, 1,058 long by 298 wide. Testes 26-40 (32 ± 5; 8) in number, 12-36 (21 ± 6.1; 9; 27) long by 35-73 (50 ± 9.1; 9; 27) wide in mature proglottids; one row deep in cross-section, decreasing in number with increasing maturity, distributed in two columns; poral column extending from the anterior margin portion of proglottid to genital pore moving towards median along anterior margin of cirrus sac; aporal column extending from anterior margin of proglottid to level of posterior margin of cirrus sac or slightly beyond. Vas deferens extending

posteriorly from ootype region to posterior margin of proglottid expanding anteriorly into a large external seminal vesicle that extends from posterior margin of proglottid to the cirrus sac before entering at its distal end. Internal seminal vesicle present. Cirrus sac inverted L-shape, thick walled, 175-317 (250 ± 41.7; 15) long by 47-97 (72 ± 13.4; 15), containing coiled cirrus. Cirrus armed. Ovary H-shaped in dorsoventral view (Fig. 20 B), bilobed in cross-section (Fig. 19 E), 309-643 (464 ± 126.7; 6) long by 124-242 (186 ± 46.1; 6) wide, lobulated. Vagina absent. Genital pores lateral, irregularly alternating, 61-76% (68 ± 4.7; 15) of proglottid length from posterior end. Genital atrium reflexed posteriorly when cirrus is everted. Uterus thin-walled, weakly developed in mature proglottids, extending from ootype along lateral margin to genital pore. Uterus extending along entire length of gravid proglottids; uterine duct not observed. Vitellaria follicular, medullary, in two lateral fields, multiple follicles in cross-section, extending from anterior of proglottid to anterior portion of ovarian lobes sometimes to posterior margin of proglottid in very mature worms, poral field slightly interrupted by cirrus sac; vitelline follicles 4-21 (12 ± 5.1; 6; 18) long by 14-59 (44 ± 14.5; 6; 18) wide. One dorsal and one ventral pair of excretory ducts. Eggs examined in whole mounts. Eggs contained in cocoons. Cocoon boundaries indistinct, not measured. Eggs subspherical. Oncospheres 10-13 (12.2 ± 1.1; 2; 10) long by 9-12 (10.5 ± 0.8; 2; 10) wide.

Taxonomic Summary

Type host: *Rhinoptera bonasus* (Mitchill), Cownose ray (Rhinopteridae, Myliobatiformes).

Additional Hosts: None.

Site of Infection: Spiral Intestine.

Type Locality: Horn Island, Mississippi, Gulf of Mexico, U.S.A.

Additional Localities: Chandeleur Islands, Louisiana, Gulf of Mexico, U.S.A.

Type Specimens: Holotype (USNPC No. 00000), five paratypes, (four whole mounts, one cross-section series) (USNPC Nos. 00000); five paratypes (LRP Nos. 00000); five paratypes (KUNHM Nos. 00000).

Etymology: This species is named in honor of Sarah (Sedlock) Call, the author's wife.

Remarks

The presence of two distinct regions of the strobila, i.e., an anterior region consisting of laterally expanded, non-reproductive proglottids, forming a trough, and a reproductive region with internal reproductive anatomy, as well as the absence of a vagina, clearly identify this species as belonging to the genus *Eniochobothrium*.

Eniochobothrium sedlockae can be distinguished from all other species of *Eniochobothrium* by the shape of its cirrus sac. In *E. sedlockae* the cirrus sac is in the form of an inverted L-shape while in all other species the cirrus sac is U-shaped. *Eniochobothrium sedlockae* can be further distinguished from *E. qatarense* and *E. gracile* based on its shorter total length (1,366-3,191 vs. 3,250-5,650 and 3,500-12,000, respectively). The maximum width *E. sedlockae* is less than that of *E. qatarense* (218-340 vs. 600-850). *Eniochobothrium sedlockae* is difficult to distinguish from *E. gracile* because the original description of the latter species provided by Shipley and Hornell (1906) lacks detail. Shipley and Hornell (1906)

described and illustrated *E. gracile* as possessing a short neck of three segments; a region lacking in *E. sedlockae*. In addition to cirrus sac shape, *E. sedlockae* can be further distinguished from *E. euaxos* and *E. overstreeti* by the extent of the vitellaria: in *E. sedlockae* they extend anterior to the genital pore, while in *E. euaxos* and *E. overstreeti* they do not.

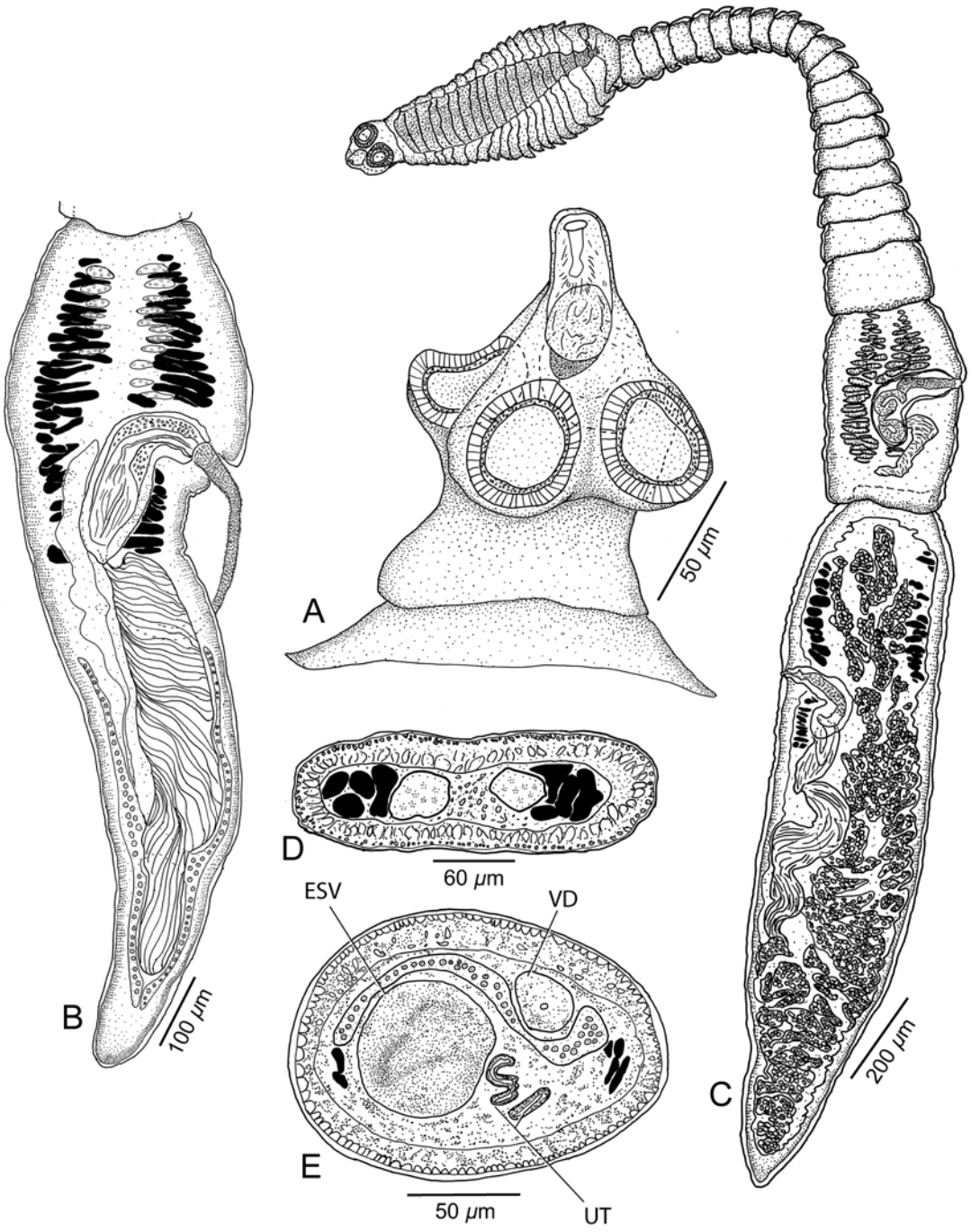

Figure 19. Line drawings of *Eniochobothrium sedlockae* n. sp. (A) Scolex (USNPC No. 00000). (B) Mature proglottid (USNPC No. 00000). (C) Whole worm (USNPC No. 00000). (D) Cross-section anterior to genital pore (USNPC No. 00000). (E) Cross-section at level of ovarian bridge (USNPC No. 00000). Abbreviations: ESV, external seminal vesicle; VD, vas deferens; UT, uterus.

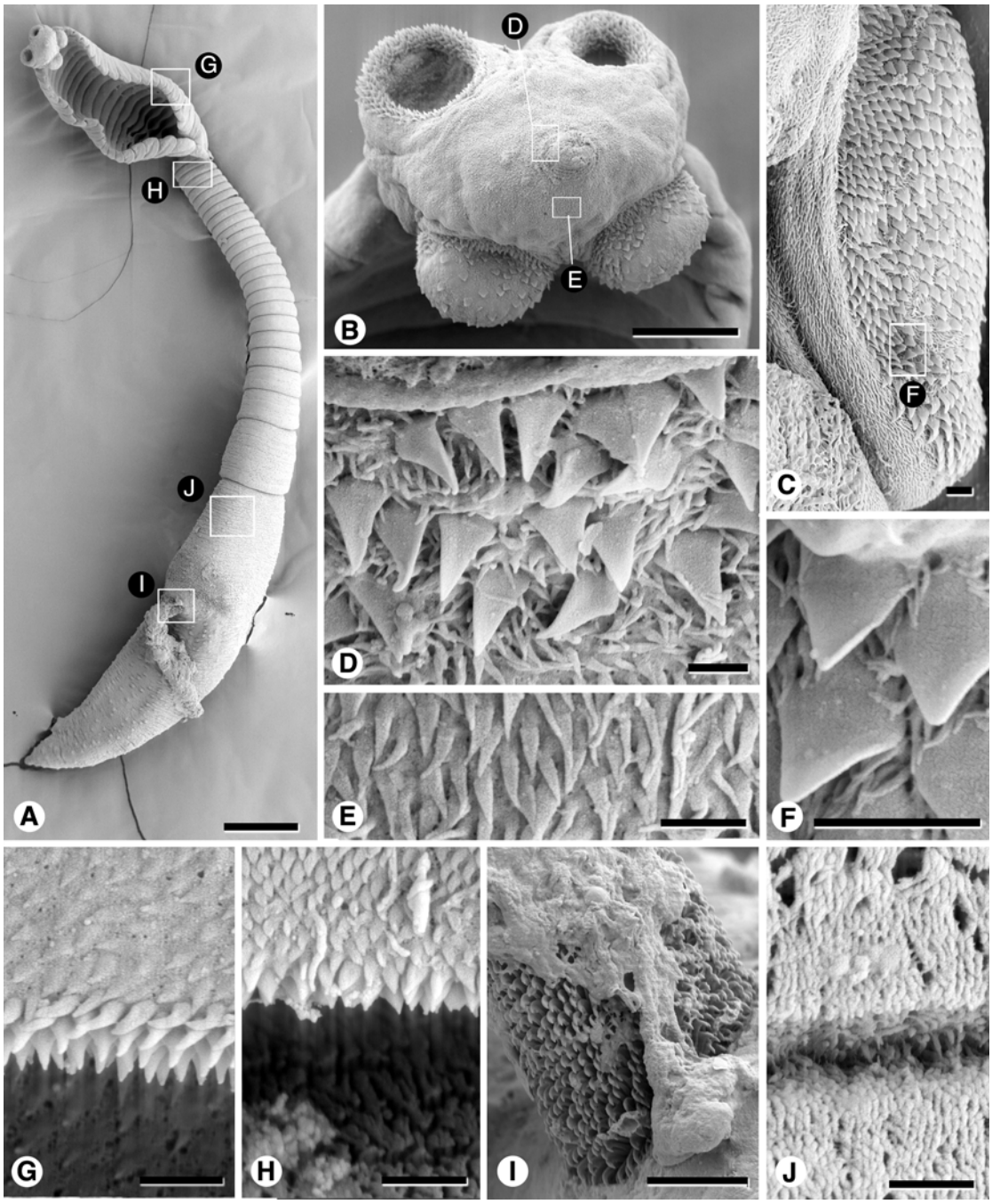

Figure 20. Scanning electron micrographs of *Eniochobothrium sedlockae* n. sp. (A) Whole worm; boxes indicate where Fig. 20G-I were taken. (B) Scolex; boxes indicate where Fig. 20D-E were taken. (C) Acetabula; box indicates where Fig. 20F was taken. (D) Apical modification of scolex proper. (E) Scolex proper. (F) Distal acetabular surface. (G) Non-reproductive strobila surface. (H) Immature proglottid surface, near trough. (I) Cirrus spines. (J) Reproductive proglottid surface. Scale bars: A, 100 µm; B, 20 µm; I, 10 µm; C, F, 2 µm; D, E, G, H, J, 1 µm.

Polypocephalus patricki n. sp.

(Figs. 21 A-G, 22 A-H)

Description

Based on 24 whole mounts, one specimen cross-section series, two lactophenol preparations of eggs, and three specimens prepared for SEM.

Worms 525-1,722 (969 ± 309.2; 24) long, maximum width at level of scolex. Proglottids 3-5 in number, acraspedote, anapolytic. Scolex 152-245 (195 ± 23.5; 24) long by 115-256 (206 ± 35.8; 24) wide, bearing four acetabula. Acetabula sucker-like in form, sessile, 46-76 (61 ± 8.4; 24; 45) long by 40-78 (60 ± 9.2; 24; 45) wide. Apical modification of scolex proper with expandable aperture at apex housing apical organ. Apical organ divided into 16 tentacles. Tentacles completely invaginable into tentacular pouch. Tentacular pouch (with invaginated tentacles) 93-163 (129 ± 21.1; 18) long by 91-171 (132 ± 21.7; 18) wide. Scolex proper covered with elongate pointed filitriches (Fig. 22 E). Rims and distal acetabular surface covered with spathate (blade-like, *sensu* Jensen, 2001) spinitriches and elongate pointed filitriches (Fig. 22 H). Apical modification of scolex proper covered with ivy leaf-shaped spinitriches and long filitriches (Fig. 22 C). Tentacles covered with small tubercles suggesting glandular surface (Fig. 22 D). Strobila covered with long filitriches (Fig. 22 F). Cephalic peduncle absent.

Immature proglottids 0-2 in number, wider than long, two most posterior immature proglottids 10-131 (41 ± 27.0; 24; 51) long by 93-167 (132 ± 17.9; 24; 51) wide. Mature proglottids 0-1 in number, square to longer than wide, posterior-most

mature proglottid 120-247 (179 ± 35.1; 15) long by 89-175 (129 ± 23.7; 15) wide. Gravid proglottids 1-2 in number, 218-771 (409 ± 135.8; 35) long by 119-233 (164 ± 26.4; 35) wide. Posterior third of terminal proglottid curved toward genital pore at less than or equal to 90 degrees (Fig. 22 A). Testes six in number, 9-37 (18 ± 5.6; 45) long by 37-99 (59 ± 15.2; 45) wide in mature proglottids, in single column in dorsoventral view, one row deep in cross-section, slightly displaced to lateral margin of proglottid by uterus, degenerated in gravid proglottids. Vas deferens observed only in gravid proglottids; coiling anterior to cirrus sac. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac containing coiled cirrus; cirrus unarmed. Ovary in four unequal lobes, H-shaped in dorsoventral view (Fig. 21 C), X-shaped in cross-section (Fig. 21 F), 40-94 (60 ± 13.1; 15) long by 58-116 (88 ± 17.8; 15) wide, degenerated in older gravid proglottids, ovarian margins smooth. Vagina thin-walled, opening into genital atrium at same level as cirrus sac; vaginal sphincter absent; seminal receptacle not observed. Genital pores lateral, irregularly alternating, 12-33% (22 ± 6.1; 15) of proglottid length from posterior end in mature proglottids. Uterus slightly lateral in mature proglottids, extending from ootype to posterior margin of most anterior testis in mature proglottids, extending entire length in gravid proglottids; uterine duct not observed; uterine pore not observed. Vitellaria follicular, medullary, in two lateral columns in dorsoventral view, 1-2 columns deep in cross-section on each lateral margin, extending from posterior margin of proglottid to slightly overlapping third testis from anterior end, uninterrupted by ovary; postovarian vitelline follicles present; vitelline follicles 6-28 (14 ± 4.9; 45) long by 8-

45 (21 ± 8.5; 45) wide. One pair of excretory ducts present. Eggs in younger gravid proglottids single, possessing bipolar filaments, oncospheres 13-19 (16 ± 1.5; 2; 26) in diameter (Fig. 21 D); eggs in older gravid proglottids contained in fibrous matrix, oncospheres 22-30 (26 ± 1.9; 2; 25) in diameter (Figs. 21 F, 22 G).

Taxonomic Summary

Type host: *Rhinoptera bonasus* (Mitchill), Cownose ray (Rhinopteridae, Myliobatiformes)

Additional Hosts: None.

Site of Infection: Spiral Intestine.

Type Locality: Ship Island, Mississippi, Gulf of Mexico, U.S.A.

Additional Localities: Horn Island, Mississippi, Gulf of Mexico, U.S.A.

Type Specimens: Holotype (USNPC No. 00000), eight paratypes (seven whole mounts, one cross-section series) (USNPC Nos. 00000); six paratypes (LRP Nos. 00000); six paratypes (KUNHM Nos. 00000); six paratypes (HWML Nos. 00000).

Etymology: This species is named in honor of the author's father, Patrick Call, for his encouragement and support of the author's education.

Remarks

The four acetabula in the form of suckers, an apical organ divided into tentacles, and few testes arranged in a single column located anterior to the ovary clearly identify this species as belonging to the genus *Polypocephalus*.

Currently, 26 species of *Polypocephalus* are recognized as valid (see Jensen, 2005). *Polypocephalus patricki* can be distinguished from *P. coronatus* Subhapradha, 1951, *P. indicus* Desmukh, Jadhav and Shinde, 1982, *P. lintoni* Subhapradha, 1951, *P. moretonensis* Butler, 1987, *P. rhynchobatidis* Subhapradha, 1951, *P. saoudi* Hassan, 1982, *P. vesicularis* Yamaguti, 1960, and *P. vitellaris* Subhapradha, 1951, based on the possession of six rather than four testes, and from *P. maharashtra* Desmukh, Jadhav and Shinde, 1982 which possesses 12 testes. *Polypocephalus ratnagiriensis* Jadhav, Shinde, and Sarwade, 1986, and *P. rhinobatidis* Subhapradha, 1951 differ from *P. patricki* in proglottid number, ranging from 10 to 250 proglottids (10-16 and 250, respectively), while *P. patricki* has a maximum of five proglottids. The maximum total length of *P. patricki* is less than 2 mm, while *P. digholensis* Desmukh, Jadhav and Shinde, 1982, *P. elongatus* (Southwell, 1912) Jensen, 2005, *P. karbharii* Desmukh, Jadhav, and Shinde, 1982, *P. katpurensis* Shinde and Jadhav, 1981, *P. prathibhai* Desmukh, Jadhav and Shinde, 1982, *P. pulcher, P. radiatus* Braun, 1878, *P. ratnagirensis, P. rhinobatidis,* and *P. singhii* Jadjav and Shinde, 1981 are all greater than 5 mm in total length. Also, *P. patricki* has a shorter ovary (40-94 vs. 890, 310, 800, and 200, respectively) than *P. karbharii, P. katpurensis, P. pratibhai*, and *P. singhii*. *Polypocephalus patricki* possesses a smaller scolex than *P. alii* Shinde and Jadhav, 1981, and *P. djiddensis* Jadhav and Shinde, 1989 (152-245 x 115-256 vs. 410 x 430 and 1,635 x 1,435, respectively). *Polypocephalus patricki* differs from *P. digholensis* and *P. elongatus* in its shorter mature proglottids

(maximum length 247 vs. 290 and 400, respectively) and *P. patricki* from *P. pulcher* in the number of tentacles (16 vs. 14).

Distinguishing *P. patricki* from *P. affinis* Subhapradha, 1951 , *P. bombayensis* Shine, Dhule and Jadhav, 1992, *P. medusia* (Linton, 1890) Southwell, 1925, *P. radiatus* Braun, 1878, and *P. thapari* Shinde and Jadhav, 1981 is more difficult due to the cursory nature of the original descriptions. *Polypocephalus patricki* possesses unpaired tentacles, whereas *P. affinis* has paired tentacles. Further distinguishing *P. patricki* from *P. affinis* is the fact that *P. affinis* is longer (total length 3,640 vs. 525-1,722) and possesses a greater number of proglottids (21 proglottids in an illustration of incomplete worm vs. maximum of five proglottids). In *P. bombayensis*, the cirrus sac is oriented horizontally and the genital pore is illustrated in the anterior third of the proglottid, whereas the cirrus sac in *P. patricki* is slightly angled anteriorly and is located in the posterior third of the proglottid. *Polypocephalus medusia* possesses more proglottids than *P. patricki* (10-15 based on illustrations by Linton [1890] vs. 3-5). Also, *P. medusia* can reach a maximum length of 6,000 whereas in *P. patricki* the largest specimen measures 1,722. *Polypocephalus thapari* is distinguished from *P. patricki* by an ovary that is elongated laterally in the posterior of the proglottid whereas in *P. patricki* the ovary is H-shaped. Furthermore, in *P. thapari* the genital pore is located at the level of the third testis (counting from posterior) whereas in *P. patricki* the genital pore is located posterior to all six testes.

The species that most closely resembles *P. patricki* is *P. helmuti* Jensen, 2005. *Polypocephalus patricki* is distinguished from *P. helmuti* by the extent of the

vitellaria. In *P. patricki* the vitellaria extend from the posterior proglottid margin to the posterior margin of the fourth testis (counting from posterior), never overlapping the fourth testes, whereas in *P. helmuti* the vitellaria extend from the posterior proglottid margin to the fourth testis, (counting from posterior) overlapping and extending in some specimens almost to anterior proglottid margin. Also, *P. patricki*, in general, possesses a greater number of total proglottids and greater number of gravid proglottids.

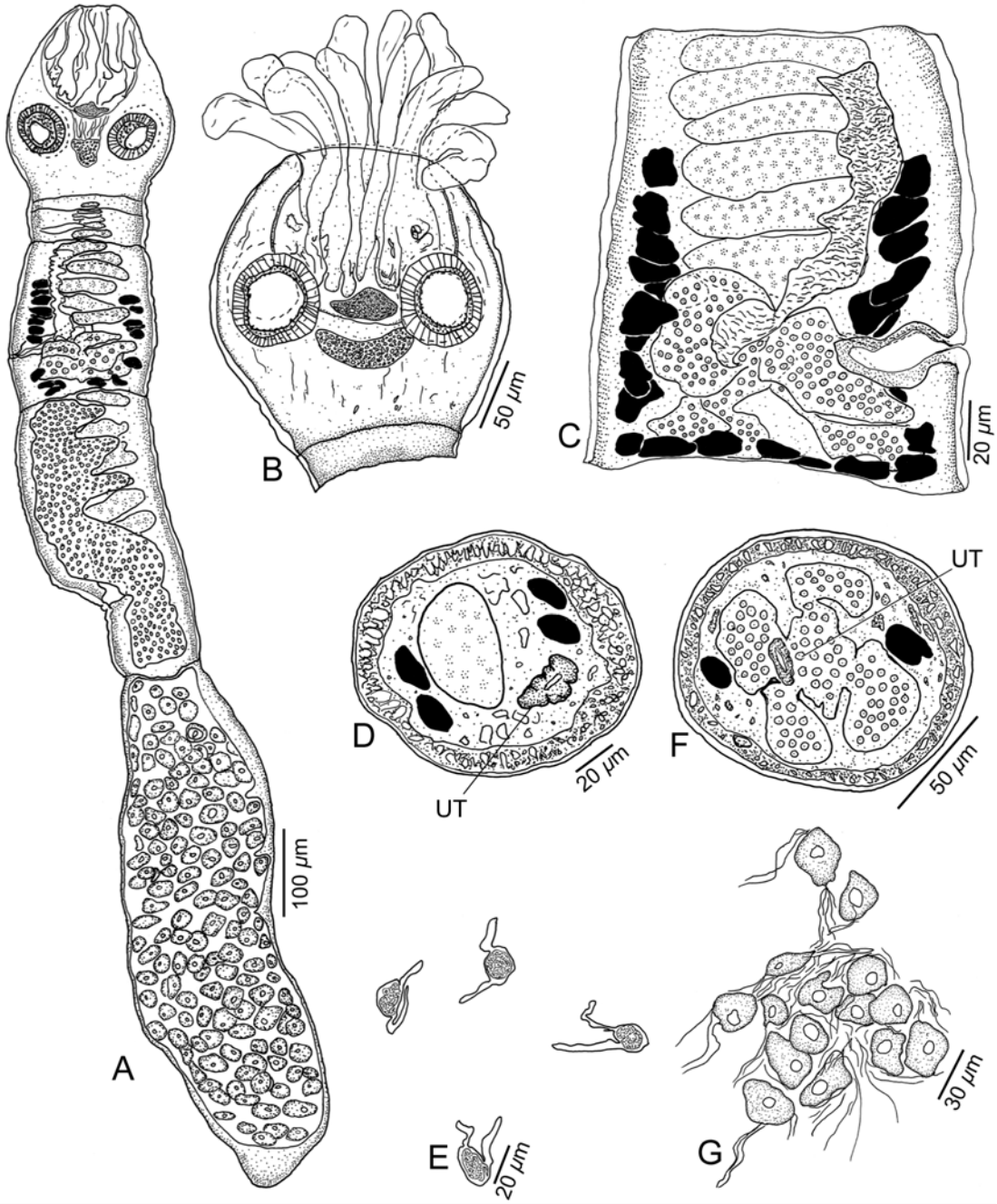

Figure 21. Line drawing of *Polypocephalus patricki* n. sp. (A) Whole worm (USNPC No. 00000). (B) Scolex (USNPC No. 00000.) (C) Mature Proglottid (USNPC No. 00000.) (D) Cross-section anterior to genital pore (USNPC No. 00000). (E) Eggs from younger gravid proglottids. (F) Cross-section at level of ovarian bridge. (G) Eggs from older gravid proglottids. Abbreviation: UT, uterus.

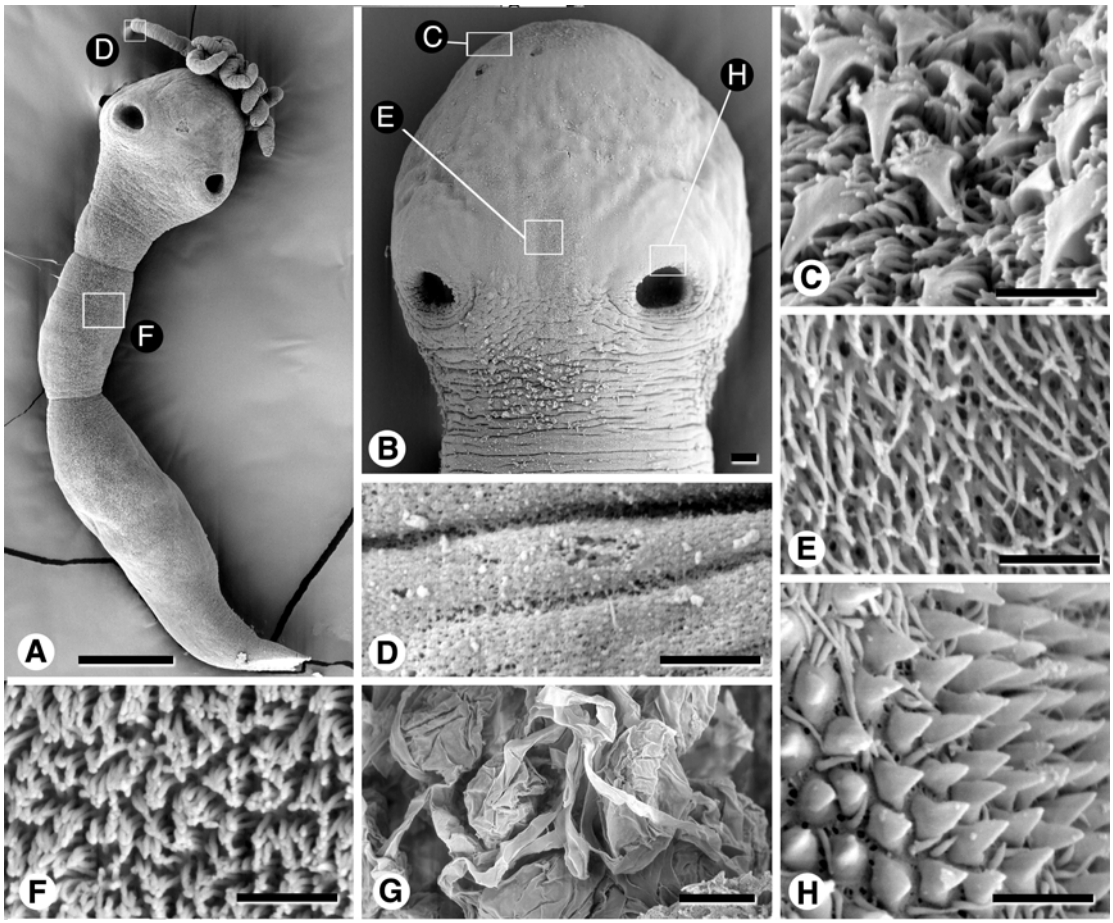

Figure 22. Scanning electron micrographs of *Polypocephalus patricki* n. sp. (A) Whole worm; boxes indicate where Fig. 22D-F were taken. (B) Scolex; boxes indicated where Fig. 22 C, E, and H were taken. (C) Apical modification of scolex proper. (D) Tentacle surface. (E) Scolex proper. (F) Strobila surface. (G) Eggs. (H) Distal acetabular surface. Scale bars: A, 100 µm; B, G, 10 µm; C-F, H, 1 µm.

Discussion

As a result of this study, the number of cestode species known to parasitize *Rhinoptera bonasus* throughout its range has increased from 13 to 19. *Rhinoptera bonasus* is reported as a host for *Echinobothrium fautleyae* for the first time. The distribution of seven species (i. e., *Mecistobothrium brevispine*, *Rhinoptericola megacantha, Echinobothrium bonasum, Echinobothrium fautleyae, Dioecotaenia cancellata, Rhodobothrium paucitesticulare, Tylocephalum bonasum*) is expanded to include the Gulf of Mexico. Six species known to parasitize *R. bonasus* outside the Gulf of Mexico were not found.

In comparison to the southern stingray, *Dasyatis americana* Hildebrand and Schroeder, a host with a similar geographic distribution to that of *R. bonasus*, the known tapeworm species encountered was similarly diverse. Approximately 15 species are known from *D. americana* (Campbell, 1969, 1975; Mayes & Brooks, 1981; Ruhnke, 1994; Palm 2004), as compared to the 19 now known to parasitize *R. bonasus*. As was the case for *R. bonasus*, it appears that the complete fauna of *D. americana* from a single locality has not been described. A focused survey of the tapeworms of *D. americana* of this type might increase the faunal diversity much like this study increased known cestode diversity and geographic distributions of cestodes in *R. bonasus*.

Of the five new species described as part of this study, three belong to the lecanicephalidean genera *Eniochobothrium* and *Polypocephalus*. None of the three valid, previously known species of *Eniochobothrium* and the 26 valid species of *Polypocephalus* (see Jensen, 2005) had been reported from *R. bonasus*. Species of *Eniochobothrium* parasitize other species of *Rhinoptera*, *R. javanica* from Sri Lanka,

Rhinoptera adspersa Müller and Henle from the Arabian Gulf, and *Rhinoptera* sp. from northern Australia in the Pacific Ocean (Shipley and Hornell, 1906; Al Kawari, 1994; Jensen, 2005, respectively). This study expands the distribution of the genus *Eniochobothrium* to include the Gulf of Mexico. It is likely that additional species of *Eniochobothrium* parasitize species of *Rhinoptera* not previously examined (e.g., *R. neglecta* and/or *R. marginata*). This is the first instance of congeners of *Eniochobothrium* parasitizing the same host species (see Table 4). It is interesting to note, while *E. overstreeti* was found in high intensities in several cownose ray specimens, a larger species, *E. sedlockae*, was only found in low numbers. Interestingly, Jensen (pers. comm.) found a second species of *Eniochobothrium* in addition to *E. euaxos*, albeit in low numbers, from *Rhinoptera* sp. from northern Australian waters. Two species of *Eniochobothrium* found in each species of *Rhinoptera* may be a pattern seen in future studies.

The genus *Polypocephalus* parasitizes a variety of hosts including guitarfishes and eagle rays (see Jensen, 2005). However, the genus has only been reported from one species of *Rhinoptera* other than *R. bonasus*. Jensen (2005) described *P. helmuti* from *Rhinoptera* sp. from northern Australia. *Polypocephalus patrick*i is similar in morphology to *P. helmuti*. While there exist morphological features that allow these two species to be distinguished, the differences identified at this point are rather subtle. In addition to the morphological characteristics used to distinguish the species, examination of voucher specimens of *P. helmuti* showed morphological variation that might be of taxonomic value. The posterior most gravid proglottid in most specimens was strongly hooked, that is, curved past 90º, whereas in *P. patricki*, in all specimens examined the curvature was

always less than 90º. It would be interesting to compare molecular sequence data for these two taxa (e.g., 28S LSU rDNA or COI) to investigate the degree of sequence similarity between these morphologically similar species. A genetic analysis of the two species, as well as a careful examination of additional collections of *P. helmuti* promise to shed light on these identities. Examination of tapeworms from other species of *Rhinoptera* may also add to our understanding of these morphologically very similar species with the potential of discovering additional species of *Polypocephalus*.

Of the species known to parasitize *R. bonasus*, six were not found in this study. Among them are those of the order Trypanorhyncha: the species reported as *Zygorhynchus* sp. and *Nybelinia* sp. (USNPC No. 4821). Linton (1897; 1901) originally reported these species,as *Tetrarhynchus robustum* Linton, 1890 (based on 2 specimens) from *R. bonasus* at Woods Hole, Massachusetts, U.S.A. This represented a new host record for *T. robustum*, which was originally described from *Dasyatis centroura* (Mitchill). Palm (1999) transferred *T. robustum* to *Heteronybelinia* Palm, 1999 to become *Heteronybelinia robusta* (Linton, 1890) Palm, 1999. Palm (2004) later examined the 2 voucher specimens deposited by Linton from *R. bonasus* and placed one specimen each in the genera *Zygorhynchus* Beveridge and Campbell, 1988, and *Nybelinia* Poche, 1926. Two species of tetraphyllideans, *Glyphobothrium zwerneri* and *Dioecotaenia campbelli*, and two species of lecanicephalideans, *Tylocephalum brooksi* and *T. pingue* were also absent from cownose rays in the Gulf of Mexico. *Glyphobothrium zwerneri* has only been reported from the east coast of the United States in Chesapeake Bay, Virginia, U.S.A. *Dioecotaenia campbelli*, and *T. brooksi,* and *T. pingue* are discussed in detail below.

Of the only known members of the dioecious genus *Dioecotaenia*, *D. campbelli* and *D. cancellata,* only one, *D. cancellata* was found in this study. No records exist of both species infecting a single host specimen. *Dioecotaenia campbelli* was described as possessing 24 bothridial loculi (Mayes & Brooks, 1981) and *D. cancellata* was described as possessing 21 bothridial loculi (Linton, 1890; Schmidt, 1969). No variation in loculi number has been reported for either species. Examination of the voucher material deposited by Schmidt (1969) of *D. cancellata* showed unexpected variability in bothridial loculi number, exhibiting a range of 20-22 loculi (see Fig. 6). This variation was also observed in the species collected in this study. Examination of *D. campbelli* was limited to two type specimens and no variability in bothridial loculi number was evident both possessed 24 loculi (see Fig. 5). However, in light of the variation in bothridial loculi seen in *D. cancellata*, it is suspected that a similar amount of variation might be possible in the number of bothridial loculi in *D. campbelli*. These two species are distinguished only by number of bothridial loculi and egg and embryo size, which can vary with maturity. Were the number of bothridial loculi to overlap, the species boundaries would have to be reexamined.

Loculi arrangement in *Dioecotaenia* has received considerable attention in the literature. Schmidt (1969) and Mayes & Brooks (1981) noted that the arrangement of loculi could be viewed as three columns of seven in *D. cancellata* or three columns of eight in *D. campbelli*. Brooks (1982) added complexity to the discussion of loculi arrangements by suggesting a formula for the loculi arrangement of *Dioecotaenia* as, $3(X) = (X - 2) + (2X + 2)$, where X is the number of loculi in a single column. On the left side of the equation 3(X) equals the total number of loculi, while on the right hand side

(X – 2) predicts the number of central loculi and (2X + 2) predicts the number of marginal loculi. For *D. cancellata* X = 7 and for *D. campbelli* X = 8 as expressed by Schmidt (1969) and Mayes & Brooks (1981). Brooks (1982) evaluated possible bothridial forms by increasing and decreasing X. Thus, one hypothesized bothridial loculi arrangement for a yet to be discovered species, could be expressed as 3(9) =(9-2) +[(2)(9) + 2] or 27 total loculi (i.e., 7 central loculi + 20 marginal loculi) (Brooks, 1982). Brooks (1982) tempers his prediction of bothridial forms as a "bold hypothesis" which may be "tested by new collections"(p. 56, lns. 25-26). Based on the interpretation of the voucher specimens and new collections of *D. cancellata* this algebraic assessment of bothridial arrangement no longer holds due to the variability in bothridial loculi number.

The genus *Duplicibothrium* appears to be restricted to *Rhinoptera*. In addition to *R. bonasus*, species of *Duplicibothrium* have been described from *R. steindachneri* (Ruhnke et al., 2000). To date, *R. bonasus* was known to be parasitized by a single species of *Duplicibothrium*, while two species, *D. cairae* and *D. paulum*, were found to parasitize *R. steindachneri*. This study adds two new species, *D. karenae* and *D. mergacephalum*, to the genus, increasing the total number of *Duplicibothrium* species parasitizing *R. bonasus* to three. The addition of these species added greatly to the morphological diversity of scolex forms in this genus (see Fig. 12). *Duplicibothrium mergacephalum* possesses as many as 33 rows of three loculi whereas *D. minutum* possesses 7-8 rows of a single loculus. Furthermore, greater variation is now seen in the number of loculi comprising the posterior row with as few as three in *D. mergacephalum* and *D. paulum*, an intermediate number of five in *D. karenae*, and seven in *D. cairae* and

D. minutum. It appears that two or three congeners infecting a single species of cownose ray might be the rule, rather than the exception.

As discussed by Ruhnke et al. (2000) the species described as *Echeneibothrium javanicum* Shipley & Hornell, 1906 from *Rhinoptera javanica* bears a strong resemblance to *Duplicibothrium* species. The illustration of this species by Shipley & Hornell (1906) shows the bothridia of *E. javanicum* as possessing an apical loculus, seven rows of three loculi, and a posterior row of seven loculi (Shipley & Hornell, 1906, Plate IV, figs. 51-54). While their cross-section of the scolex (see Shipley & Hornell, 1906, fig. 56) shows a compact posterior region, their figures 52 and 53 show separated, stalked, bothridia rather than bothridia fused in back-to-back pairs, which lead Ruhnke et al. (2000) to refrain from transferring this species to the genus *Duplicibothrium*. Transfer of *E. javanicum* to *Duplicibothrium* is supported here. Further examinations of tapeworms of *R. javanica* and other species of *Rhinoptera* are likely to reveal additional species of *Duplicibothrium*.

Familial boundaries of non-hooked, loculated tetraphyllideans to which *Dioecotaenia* and *Duplicibothrium* belong are not clearly defined, and knowledge of sister group relationships is often lacking. Traditionally, the non-hooked loculated tetraphyllideans have been recognized in two families (Euzet, 1994), the Dioecotaenidae, which includes *Dioecotaenia*, and the Phyllobothriidae, which, among others, includes *Duplicibothrium*. Within the Phyllobothriidae, *Duplicibothrium* was placed in the subfamily Rhinebothriinae along with the other facially loculated taxa parasitizing rays (e.g., *Rhinebothrium* Linton, 1889; *Caulobothrium* Baer, 1948; *Rhabdotobothrium* Euzet, 1953). Brooks & Barriga (1995) erected the family Serendipidae for *Duplicibothrium,*

Glyphobothrium, and *Serendip*. The family was distinguished based on its degree of bothridial fusion, testes arranged in two dorsoventral fields and distributed into ovarian field, a digitiform ovary radiating from a central isthmus, and vitelline fields that converge dorsally, except dorsal to the cirrus sac and ovary (Brooks & Barriga, 1995). *Duplicibothrium karenae, D. mergacephalum*, and the redescribed *D. minutum* share these features proposed for the family. Additionally, Brooks & Barriga (1995) considered the genus *Dioecotaenia* to be closely related to the Serendipidae because of the anterior position of the cirrus sac and the absence of bothridial apical suckers. Ruhnke et al. (2000) add further evidence of this close relationship of *Dioecotaenia* to the Serendipidae in the form of microthrix data. The scanning electron microscopic data presented here for *D. minutum, D. karenae,* and *D. mergacephalum* show short filitriches on all surfaces and no spinitriches on any surface. This is consistent with the microthrix pattern described from *D. cairae, D. paulum, G. zwerneri,* and *Dioecotaenia* sp. (Ruhnke et al. 2000; Caira et al., 1999). (Spinitriches were referred to as the large class of microtriches in Ruhnke et al. [2000]). According to Ruhnke et al. (2000), ultrastructurally distinct spinitriches (absent in these taxa) are thought to be plesiomorphic for tetraphyllideans and absence of these structures is derived. Unfortunately, the surfaces of the specimens in this study of *D. cancellata* were disrupted and the microthrix pattern of this species could not be observed.

As mentioned in the introduction, Smith & Merriner (1987) suggested that semi-isolated populations of *R. bonasus* exist. Six species of tapeworms found parasitizing *R. bonasus* in other localities were not found in *R. bonasus* from the Gulf of Mexico. If these species are indeed restricted to cownose rays from other localities, a detailed survey

of *R. bonasus* throughout its range, including data on prevalence and intensity of all species might lend support to the hypothesis of isolated populations of *R. bonasus*. Also, if these populations are indeed "semi-isolated," unrecognized cestode diversity might be discovered in other populations. In addition, degrees of similarity of tapeworm faunas parasitizing R. *bonasus* in these regions might elucidate migratory patterns. In coastal waters of Senegal and Guinea, only two species of cestodes, *Duplicibothrium* cf. *minutum* and *Duplicibothrium* n. sp. have been reported from *R. bonasus* by Healy, (2006). Conspecificity of these species with those identified as part of this study remains to be investigated.

Conclusions about the geographic range of tapeworm species or the utility of distinguishing among populations of cownose rays are difficult. As mentioned before, records of tapeworms from cownose rays are mostly from the eastern coastal waters of the United States and off of South America, and are of a single species report, not complete faunal surveys for the presence of all tapeworm species. A few observations are interesting, given the relatively thorough study of tapeworms from the Gulf of Mexico. Limited distribution of tapeworm species may be indicative of limited host species range as reflected by available and/or appropriate intermediate hosts. Six species known to parasitize *R. bonasus* outside the Gulf of Mexico were not found suggesting possible restriction of their hosts' geographic distributions. For example, the absence of *Glyphobothrium zwerneri* in cownose rays from the Gulf and presence on the east coast of the U.S.A. may indicate a restricted distribution of that cestode species, supporting the suggestion of Smith and Merriner (1987) of an isolated host population the on east coast of the United States. In addition to *G. zwerneri*, two species of lecanicephalideans,

Tylocephalum pingue and *T. brooksi*, were absent from the spiral intestines collected from *R. bonasus* in this study. *Tylocephalum pingue* has only been reported from the cownose ray from the east coast of the United States, whereas *T. brooksi* was reported only from the Gulf of Venezuela. Williams and Campbell (1984) comment that the collection of *T. pingue* is more common in hosts from the warmer waters of Chesapeake Bay than from the more northern coastal waters of Rhode Island. The absence of *T. pingue* from the Gulf of Mexico suggests either that *T. pingue* is restricted to the Northwest Atlantic or that it occurs in such low prevalence to have been missed in this study. The collection of *T. brooksi* did not occur in this study, which may suggest the parasite is restricted to the Atlantic, possibly in more southern waters because it has not been collected from either the Gulf of Mexico or the east coast of the U.S.A.

While not the focus of this study, some ecological data resulted from these collections (Tables 3). Apart from prevalence and estimated intensity data for each species, an attempt was made to determine the infracommunity of each host specimen (Table 4). Assessment of competition between species is not possible without the knowledge of their direct interactions and the time at which the host became infected with each species. In addition, location and identification of every worm within the spiral intestine would have been necessary in order to rigorously test competition. At present, only general patterns of association can be discussed. In general, each cownose ray specimen hosted between three and eight tapeworm species. Within the genus *Eniochobothrium*, both species were found in the same host specimen twice, but *E. sedlockae* was always found in lower numbers than *E. overstreeti*. Interestingly, even when *E. sedlockae* was the only species of *Eniochobothrium* present (see Table 4,

Specimen Nos. 7, 9), it did not reach the same level of intensity seen in *E. overstreeti*. All three *Duplicibothrium* species were present together in a single host specimen. More often, the most prevalent species parasitizing all but one of the twelve cownose rays, *D. minutum*, is present with either *D. karenae* or *D. mergacephalum*. *Duplicibothrium minutum* is the only species within the genus found parasitizing the host without the other two (see Table 4, Specimen Nos. 2, 9, 11, 12). The author's observations suggest that *D. karenae* typically had lower intensities than did *D. minutum* in each host in which both were present, except for one instance. In general, of the three species of *Duplicibothrium, D. mergacephalum* was always present in the lowest intensities. It is interesting to note, that *D. mergacephalum* was always observed in the posterior chambers of the spiral intestine, whereas in the other species no particular pattern was apparent.

TAPEWORM LIFECYCLES AND LARVAE

Introduction

This project included investigations of possible intermediate hosts of the adult cestodes collected from *Rhinoptera bonasus*. Specifically, the variable coquina clam, *Donax variabilis* (Say), was examined to determine its contribution of larvae to the adult tapeworm fauna of *R. bonasus*. This focused investigation derived from the examination of the stomach content of one *R. bonasus* specimen while collecting the adult tapeworms. The stomach of this ray contained crushed shells of coquina clams, either those of *Donax variabilis* and/or *Donax texasianus* Philippi. *Donax variabilis* was chosen for detailed study rather than *Donax texasianus* because the former inhabits shallow water where *R. bonasus* was observed to be present, while *Donax texasianus* retreats to deeper waters during the summer (Andrews, 1977).

Donax variabilis inhabits the swash zone of beaches from Virginia to southern Florida and around the Gulf Coast to Texas (Ruppert and Fox, 1988). These clams are estimated to live anywhere from one year (Bonsdorff and Nelson, 1992) to three years (Morrison, 1971) with growth rates during the summer between 3.0-3.7 mm/month (Mikkelsen, 1985) to as much as 4.3-4.4 mm/month (Jones et al., 2004). Attempts at oxygen isotope techniques determined life spans of 3-5 months, but sample size was small and efforts were underfunded (Jones et al., 2004). Growth rings are indistinct and not useful for age determination (Wilson, 1999). Furthermore, mark recapture studies to better determine age are unfeasible due to the movement and high mortality of the species (Mikkelsen, 1985). Juvenile specimens can be less than 3 mm in shell

length (Jones et al., 2004) and adults up to 25 mm in shell length (Quitmeyer et al., 2004). The life history of this species, like its color, is particularly variable.

Feeding habit studies have shown that *R. bonasus* is generally considered to be a hard prey specialist (Smith and Merriner, 1985). Bivalves, in general, have consistently been shown as part of the diet of the species (Smith and Merriner, 1985; Blaylock, 1993). While the motivation of these diet studies was to determine the extent of feeding on commercial bivalves like the oyster, *Crassostrea virginica* Gmelin, a recent study by Collins et al. (2007) suggested that *R. bonasus* is an opportunistic generalist consuming not only bivalves as a primary food source, but crustaceans and polychaetes. The study contends that *R. bonasus* modifies its behavior to consume readily available prey.

For trypanorhynchs, identification of preadult cestodes to species is possible due to the fact trypanorhynch larvae possess adult tentacular armature primarily used to identify species (and many species have been described based on larvae alone). Larvae of the other elasmobranch tapeworm orders (i.e., Diphyllidea, Tetraphyllidea, Lecanicephalidea, Cathetocephalidea), in general, lack the distinguishing features to identify the larvae to species.

Currently, no complete lifecycle is known or has been demonstrated experimentally for any species of tapeworm parasitizing *Rhinoptera bonasus*. Hypothesized marine elasmobranch tapeworm lifecycles involve three (or two or more intermediate) or more hosts. In general, copepods act as a first intermediate host and become infected by ingesting eggs or coracidium larvae (Lauckner, 1983; Riser,

1956; Mudry and Dailey, 1971). Bivalves have also been suggested as first intermediate hosts (Cheng, 1966). The second, third, or possibly additional intermediate and/or paratenic hosts may be teleosts, other molluscs, or shrimp. For example, copepods could be eaten by a teleost fish, which in turn is ingested by a second teleost before being ingested by the elasmobranch definitive host. Mattis (1986) presented the hypothesized three-host and four-host lifecycles for two species of trypanorhynchs, *Prochristianella hispida* (Linton, 1890) Campbell and Carvajal, 1975, and *Poecilancistrium caryophyllum* (Diesing, 1850) Dollfus, 1929, respectively. For *P. hispida,* the life cycle consisted of a copepod as first intermediate host, a callianassid shrimp as second intermediate host, and three species of stingrays, *Dasyatis say* (Lesueur, 1817)*, Dasyatis sabina* (Lesueur, 1824)*,* and *D. americana* as definitive hosts. Mattis (1986) was successful in experimentally demonstrating intermediate host infections of copepods and shrimp, but was not able to infect the definitive hosts through feeding of infected shrimp. For *P. caryophyllum*, the hosts in the hypothesized life cycle consisted of a copepod as first intermediate host, a presumed teleost as second intermediate host, three possible sciaenid fishes as third intermediate hosts, and the bull shark, *Carcharhinus leucas* (Müller and Henle), as the definitive host (Mattis, 1986). Reports of larval cestodes or metacestodes are numerous (e. g., Southwell, 1924; Hutton, 1964; Cheng, 1966; Moravec et al., 1997). In the Gulf of Mexico, these reports are mainly from molluscs, crustaceans, and teleosts (e. g., Cake, 1976; 1977; 1978; Cake and Menzel 1980; Wardle, 1974; Mattis, 1986). Cake (1976) summarized information on larval cestodes from shallow-water

benthic molluscs from the northern Gulf of Mexico. Overall, 91 species of molluscs were examined for cestode larvae (Cake, 1976; Wardle, 1974). From these studies, potentially eight larval types could be attributed to adults parasitizing *R. bonasus*. Of these, one larval type, *Dioecotaenia cancellata*, was identified to species (based on number of bothridial loculi) from the gastropod, *Melongena corona* Gmelin, and two bivalves, *Anadara ovalis* (Bruguière) and *Chione cancellata* (Linné). Because of their characteristic scolex morphology as adults, the following larvae were identified to genus: *Tylocephalum* sp. from *Donax variabilis*, and an additional 32 bivalve and 16 gastropod species, *Echinobothrium* sp. from two gastropods, *Cantharus cancellarius* Conrad and *Nassarius vibex* Say, and *Polypocephalus* sp. from *Argopecten irradians* (Say). Three larval types were identified by Cake (1976) as belonging to the genus *Rhinebothrium* Linton, 1890, and one as belonging to the genus *Anthobothrium* Beneden, 1850; their scolex morphology suggests that these larval types may now be more appropriately placed in the genus *Duplicibothrium* Williams and Campbell, 1978, and *Rhodobothrium* Linton, 1897, respectively. *Duplicibothrium* type larvae (as *Rhinebothrium* sp.) were found in *Donax variabilis* and an additional 15 bivalve and 14 gastropod species (Cake, 1976). The *Rhodobothrium* type larvae (as *Anthobothrium* sp.) parasitized *Donax variabilis* and five additional bivalve species (Cake, 1976).

All larval identifications of elasmobranch tapeworms from the Gulf of Mexico have been based on morphology alone. Application of the 28S LSU rDNA gene region has shown promise as an aid in identification of tapeworm larvae. This gene

region has been suggested to exhibit sufficient variation for species level identification (Olson et al., 2001; Mariaux and Olson, 2001) and a few studies have attempted to identify cestode larvae to species using this gene region (Brickle et al., 2001; Reyda and Olson, 2003). In this study, larvae were obtained from *Donax variabilis* and adults from *R. bonasus.* Both were sequenced and overall sequence similarity was used to confirm preliminary larval identifications based on morphology and to assess the utility of this gene region for larval identifications.

Materials and Methods

Collection and Specimen Preparation

Specimens of *Donax variabilis* were collected in July of 2005, and the months of March, July, and October of 2006 from the swash zone of Ship and Horn Islands, off Ocean Springs, MS, U.S.A. using a kick net or by hand, then placed in a bucket of seawater for transport to the Gulf Coast Research Laboratory. Before dissection, total shell length (in mm) was recorded using a Mitutoyo Digital Plastic Caliper for only specimens collected in 2006. Each bivalve was carefully dissected and examined for larval cestodes under a dissecting scope. Cestode larvae prepared for examination using light and scanning electron microscopy (SEM) were relaxed in hot in tap water, fixed in 10% neutral buffered formalin, transferred to 70% ethanol for storage. For molecular study were (1) a larva was cut in half and relaxed in hot tap water, then the posterior part was fixed in 95% ethanol with the scolex preserved as a voucher for morphological study in 10% formalin, or (2) the entire larva was placed in 95%

ethanol. Whole larvae directly placed in 95% ethanol were cut and the scolex prepared as a morphological voucher just prior to sequencing. Specimens in ethanol were stored in subzero temperatures after return to the laboratory. Techniques for specimen preparation for morphology follow the protocol articulated in the Materials and Methods of the taxonomic portion of this study.

Taxon Selection

The larvae parasitizing *Donax variabilis* were preliminarily identified to genus using morphology. All the larval types recovered from *Donax variabilis* were included in the molecular analysis. Based on the larval types recovered from *Donax variabilis* the following adults from *R. bonasus* were sequenced to confirm the identity of the larvae: *Dioecotaenia cancellata, Duplicibothrium karenae* n. sp., *Duplicibothrium minutum,* and *Rhodobothrium paucitesticulare*. Opportunistically, in association with a broader survey to determine larval cestodes from intermediate hosts from the Gulf of Mexico, seven specimens consistent with the scolex morphology resembling *Duplicibothrium* were included in the analysis from two gastropod hosts, *Canthurus cancellarius* and *Polinices duplicatus*. Two additional genera of tetraphyllideans possessing subdivided bothridia were also included. the published sequence (Olson et al., 2001) of *Echeneibothrium maculatum*, Woodland 1927 (ex *Raja montagui,* North Sea, U.K. [GenBank AF382086]) was included as was the sequence of an adult specimen of *Rhinebothrium* sp. (ex *Dasyatis say*, Gulf of Mexico, U.S.A.) from a concurrent study provided by Dr. Kirsten Jensen.

Molecular Methods

Total DNA was extracted using a guanidine thiocyanate protocol. Tissue samples were placed in cell lysis buffer consisting of 100 mM NaCL, 100 mM Tris-Cl (pH 8.0), 25 mM EDTA (pH 8.0), and 0.5% SDS. Protein precipitation solution (4 M guanidine thiocyanate and 0.1 M Tris-Cl [pH 7.5]) was added to the cell lysate, centrifuged, and the protein discarded. The DNA was precipitated by adding 300 μl cold isopropanol to the supernatant and centrifuged. To this, 300 μl of 70% ethanol was added, the solution centrifuged, and dried. Genomic DNA was then hydrated with 30 μl water or 10 mM Tris-Cl (pH 8.0). Polymerase chain reaction (PCR) primers used were LSU5 (5'–TAGGTCGACCGCTGAAYTTAAGCA-3') and 1200R (5'–GCATAGTTCACCATC TTTCGG-3') (Littlewood et al., 2000). PCR products were obtained using Pure Taq Ready-To-Go PCR Beads and in a 25 μm reaction (1 μl of each primer [LSU5, 1200R], 18 μl sterile water, and 5 μl undiluted template DNA). The following thermocycling profile was used: 5 min denaturation at 94ºC; 35 cycles of 30 s at 94ºC, 45 s at 55ºC, 2 min at 72ºC; 5 min hold at 72ºC. PCR amplicons were purified using Sephadex. An approximate 1283 base pair (bp) region was sequenced with BigDye chemistry via an Applied Biosystems 3130*xl* Genetic Analyzer at the University of Kansas Natural History Museum Molecular Lab. PCR was performed using LSU5 and 1200R, as external primers and 300F (5'-CAAGTACCGTGAGG GAAAGTTG-3') and ECD2 (5'-

CTTGGTCCGTGTTTCAAGA-CGGG-3') as the internal primers (Littlewood et al., 2000).

Contiguous sequences were edited and assembled using Sequencher version 4.5 (GeneCodes Corp., Ann Arbor Michigan, U.S.A.) and a neighbor-joining tree was generated using ClustalW with default settings provided by the Kyoto University Bioinformatics Center online [URL: http://align.genome.jp/].

Results

A total number of 915 specimens of *Donax variabilis* were collected as part of this study. The size distribution of the 405 specimens of *Donax variabilis* collected in July of 2006 for which shell length was measured approached normal (Fig. 23). The smallest specimens dissected in July 2006 measured in size from 5.5 mm and to as large as 25. 4 mm. Sixty-nine percent of specimens dissected fell into the 14.5-20.5 mm size range. Three specimens collected in July 2006 were slightly larger than 25 mm (i.e., 25.2-25.4 mm) the maximal size shell length reported by Quitmeyer et al. (2004); none sampled were smaller than 3 mm. All cestode larvae recovered resided in the digestive gland of *Donax variabilis*. Cestode larvae belonged to two genera of tetraphyllidean cestodes, those consistent with the morphology of *Duplicibothrium* and of *Rhodobothrium*. Molecular diagnostics revealed that each of these larval types was identical to sequence data obtained for adults of *Duplicibothrium minutum* and *Rhodobothrium paucitesticulare*, respectively. The aligned matrix included 27 taxa

and consisted of 1283 bp. The unaligned sequence length varied from 1222 bp to 1254 bp.

Of the 915 *Donax variabilis* specimens collected, 128 (14%) were infected. Of the larvae of *Duplicibothrium minutum* collected in July 2006, 70% were in clams with the shell lengths in the range 14.5-20.5 mm (Fig. 24). Most (55%) of the relatively few larvae of *R. paucitesticulare* larvae collected (Fig. 25) were recovered from clams in the 14.5-20.5 mm size range. However, larvae of *Duplicibothrium* and *Rhodobothrium* were also collected from small (approx. 8 mm) and large (approx. 25 mm) host specimens. The greatest intensity of *Duplicibothrium* was three and for *Rhodobothrium* the greatest intensity was one. Both larval types were found in the two clams twice. More detailed information on the prevalence of each larval type is presented in Table 5; additional morphological details are presented separately for each type below. Unidentified digeneans were found in July and October of 2006 with a prevalence of 6-14% (see Table 6).

Table 5. Prevalence of larvae from *Donax variabilis* collected at Ship and Horn Islands, MS, Gulf of Mexico, U.S.A.

Larval Species	Month/Year	Prevalence
Duplicibothrium minutum		
	July 2005	9% (28 of 300)
	March 2006	4% (6 of 153)
	July 2006	17% (70 of 405)
	October 2006	14% (8 of 57)
Rhodobothrium paucitesticulare		
	July 2005	2% (7 of 300)
	March 2006	0% (0 of 153)
	July 2006	2% (9 of 405)
	October 2006	0% (0 of 57)

Table 6. Prevalence of digenean larval stages from *Donax variabilis* collected at Ship and Horn Islands, MS, Gulf of Mexico, U.S.A.

Digeneans	Month/Year	Prevalence
Sporocysts and metacercaria		
	July 2006	6% (26 of 405)
	October 2006	14% (8 of 57)

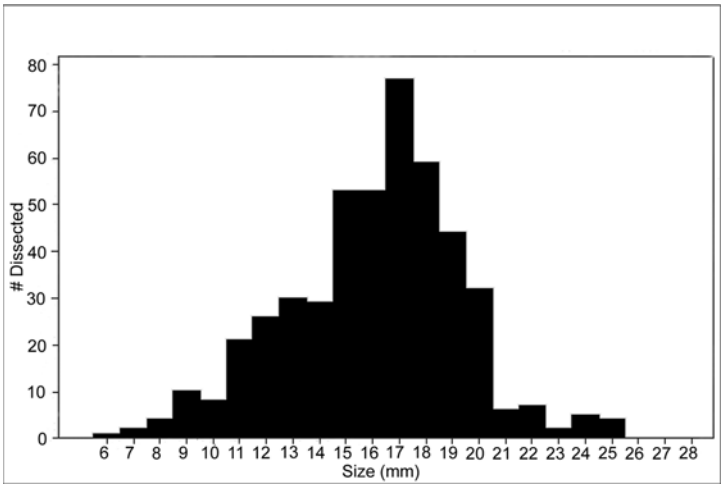

Figure 23. Histogram showing the number of *Donax variabilis* collected in July 2006 by size.

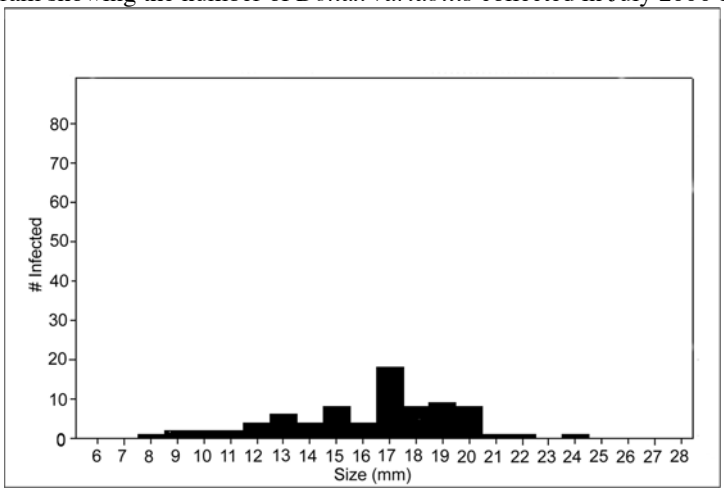

Figure 24. Histogram showing the number of *Donax variabilis* infected with larvae of *Duplicibothrium minutum* by size.

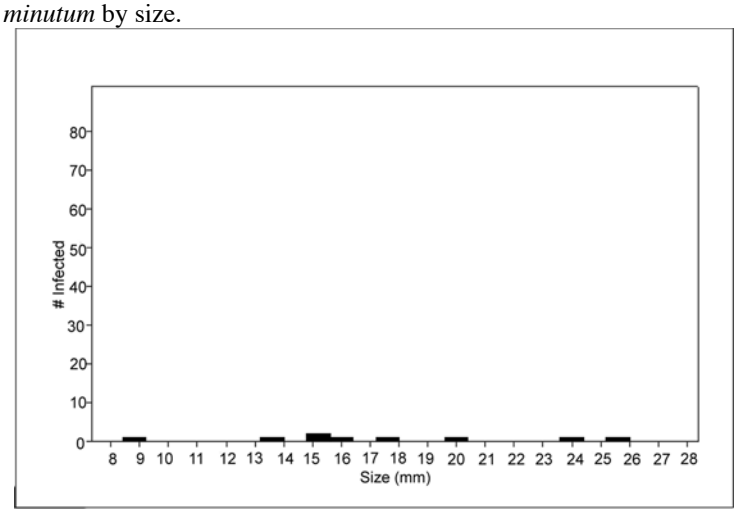

Figure 25. Histogram showing the number of *Donax variabilis* infected with larvae of *Rhodobothrium paucitesticulare* by size.

Larvae of *Duplicibothrium minutum*

(Fig. 26)

Voucher specimens deposited: Two whole mounted vouchers (USNPC Nos. 00000)

Cake (1976) reported larvae of *Duplicibothrium* from bivalves and gastropods (as *Rhinebothrium* sp.), but no larvae had been identified to species prior to this study. The bothridia of these larvae possess an arrangement of loculi similar to the adults of *Duplicibothrium minutum* but with less distinct septa. As in the adults the larvae appear to possess an apical loculus, a total of 7-8 rows of horizontal loculi (Fig. 26 B), and loculi comprising the posterior most row consistent with the arrangement in *Duplicibothrium minutum* (see Figs. 10B, 11A). Larvae were recovered that possess a more or less differentiated scolex (see Fig. 26) and larval body, but those larvae showing no apparent scolex development were also collected that appear to possess, albeit inconspicuous structure resembling an apical sucker. (Fig. 26 C). Initially, these "larvae" were interpreted as being removed from the digestive gland without scolices. Careful examination of whole digestive glands of a large number of *Donax* specimens between glass plates showed this to not be the case. Sequence data gathered for these larvae showed them to have identical sequences to larvae with a developed scolex and those of adults of *Duplicibothrium minutum*. These larvae are preliminarily interpreted as larva of *Duplicibothrium minutum* prior to scolex differentiation and are included in the prevalence and intensity data presented for *Duplicibothrium minutum*.

No microthriches were visible with scanning electron microscopy (Fig. 26 A).

Prevalence of *Duplicibothrium minutum* in *Donax variabilis* was varied between collection events (Table 5). In comparison to the other larval type present in *Donax variabilis*, *Duplicibothrium minutum* was the most prevalent (as high as 17%) and was found in each month of collection. The lowest prevalence, 4%, occurred in March (see Table 5). Intensities of *Duplicibothrium minutum* range from 1 to 3 larvae per clam.

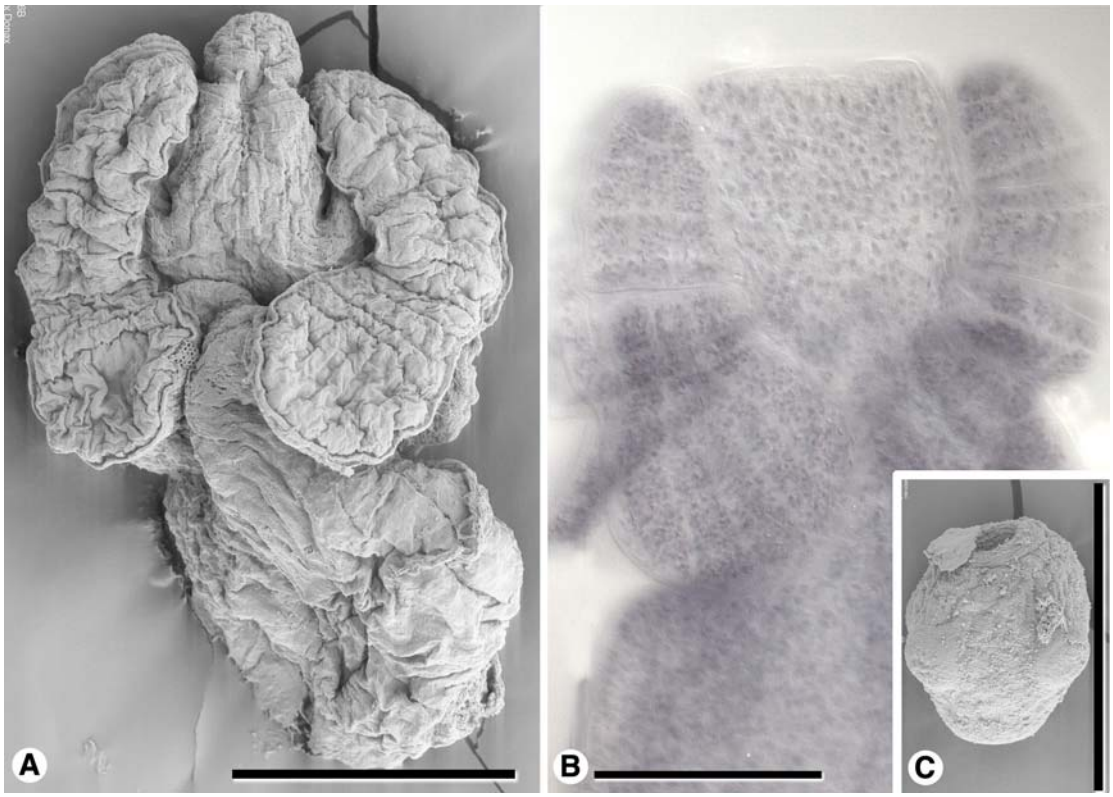

Figure 26. Larvae of *Duplicibothrium minutum* from *Donax variabilis*. (A) Scanning electron micrograph of scolex. (B) Light micrograph of scolex. (C) Undifferentiated larva. Scale bars: A-C 100 μm.

Larvae of *Rhodobothrium paucitesticulare*

(Figs. 27, 28)

Voucher specimens deposited: One whole mounted voucher (USNPC No. 00000)

These larvae are peculiar in their morphology. When initially recovered from *Donax variabilis*, larvae consist of a bulbous "blastocyst" (*sensu* Carvajal and Campbell, 1979), which surrounds the scolex and part of the undeveloped strobila, and a slender peduncle that terminates in a distinct swelling (Figs. 24, 25 A). This swelling is usually what attaches the larva to the digestive gland. Once a single outer covering is removed, an excysted larva resembling adult an adult of *Rhodobothrium* spp. unfolds. Like the adult, the larvae possess four pedicellated, unloculated bothridia, with margins that form numerous convolutions (Fig 25 D).

In the Gulf of Mexico, this larval form had been reported as *Anthobothrium* sp. (e.g., Cake, 1975, 1976, 1978; Wardle 1974). Later Campbell and Carvajal (1979) described this larval form from the bivalve *Mesodesmatum donacium* Lamarck. These latter excysted larvae were determined to be morphologically identical to adults of *Rhodobothrium mesodesmatum* from the Chilean eagle ray, *Myliobatis chilensis* Phillipi in the Pacific Ocean. Campbell and Carvajal (1979) recognized the larvae described by Cake (1976) as a member of the genus *Rhodobothrium*.

Cake illustrated the larva of *Rhodobothrium* sp. (e.g., Cake 1978, fig. 8) with an apparent invaginated scolex positioned anteriorly within the outer covering. In the

larva illustrated here (Fig. 27), the scolex is not invaginated but rather folded within the outer covering in which it is oriented posteriorly.

No scanning electron micrographs of larvae of *Rhodobothrium* have been presented previously. The outer covering of the larva is covered with short filitriches (Fig. 28 B, C). Once excysted, the scolex of the larva has four pedicellated bothridia and a short strobila. The bothridial margins form numerous convolutions. The distal bothridial surface is densely covered with short filitriches (Fig. 28 E) while the edge of the distal bothridial surface is less densely covered with short filitriches (Fig. 28 H). No microthrix were observed on the strobila (Fig. 28 G).

Larvae of *Rhodobothrium paucitesticulare* were collected only during the month of July of 2005 and 2006 and were found with a relatively low prevalence in comparison to *Duplicibothrium minutum* (0-2% vs. 4-17%, respectively) (Table 5). The intensity of these larvae was never more than one per clam.

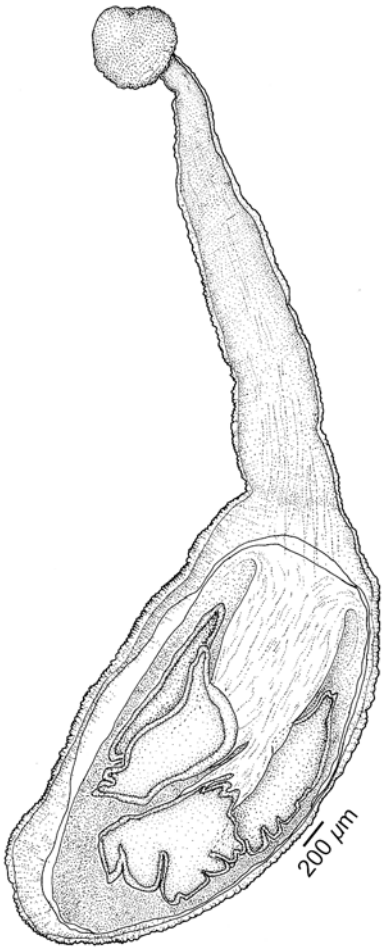

Figure 27. Line drawing of larva of *Rhodobothrium paucitesticulare* from *Donax variabilis*.

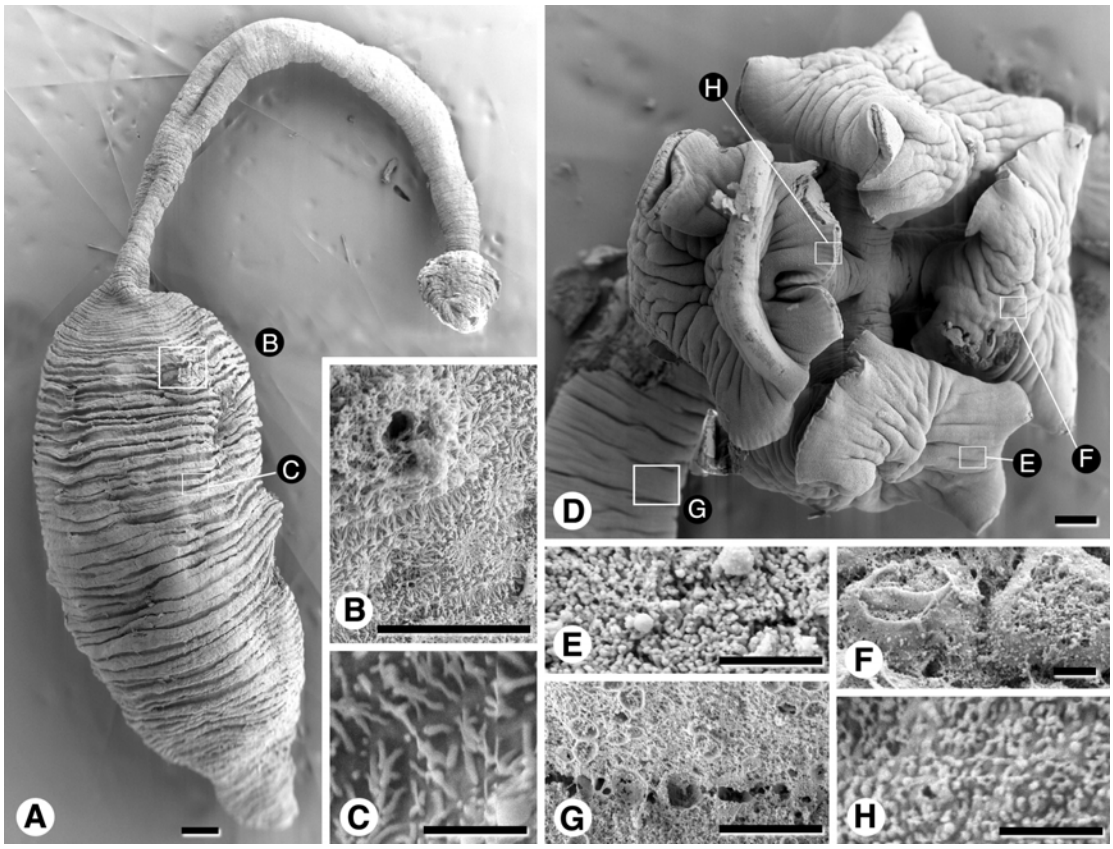

Figure 28. Scanning electron micrographs of *Rhodobothrium paucitesticulare* larvae from *Donax variabilis*. (A) Unexcysted larva; boxes indicate where Fig. 28B-C were taken. (B) Surface of outer covering. (C) Surface of outer covering. (D) Scolex of excysted larva; boxes indicate where Fig. 28E-H were taken. (E) Distal bothridial surface. (F) Anterior region of distal bothridial surface. (G) Surface of strobila. (H) Distal bothridial surface edge. Scale bars: A, 200 μm; B, 10 μm; C-H, 1 μm**.**

Molecular Results

The neighbor-joining tree resulting from the analysis formed distinct groups. None of the sequences from larvae included in this study grouped with those of adults of *Dioecotaenia*, *Rhinebothrium*, or *Echeneibothrium*. Within *Duplicibothrium*, larvae and adults formed three subgroups. The first subgroup comprised larvae identified as *Duplicibothrium* larva type II collected from two gastropod species, the moon snail, *Polinices duplicatus* (Naticidae), and *Cantharus cancellarius* (Buccinidae). Sequences of the three specimens from *Polinices duplicatus* and the four specimens from *Canthurus cancellarius* were identical; no sequence from adults from *R. bonasus* grouped with these sequences from larvae. The second subgroup comprised identical sequences of two specimens, adults of *Duplicibothrium karenae* n. sp., no larval sequence grouped with those two specimens. The third subgroup of *Duplicibothrium* comprised larvae identified as *Duplicibothrium* larva type I from *Donax variabilis* and adults of *Duplicibothrium minutum* from *R. bonasus*. Sequences of the three adult specimens and the four larvae from *Donax variabilis* and *Tellina* cf. *versicolor* were identical. These data allow confident identification of *Duplicibothrium* larva type I as *Duplicibothrium minutum*. Sequence variation between the three subgroups of *Duplicibothrium* ranged from 1 to 6% (i.e., 11-75 bp of 1283 bp).

The larvae and adults of *Rhodobothrium* formed a group distinct from that of the *Duplicibothrium* species. The group comprises sequences of four larvae collected from *Donax variabilis* and two adult specimens from *R. bonasus*. These six sequences

were identical. These data support the identities of larvae as *Rhodobothrium paucitesticulare*. The sequences of *Rhodobothrium* differs from those of *Duplicibothrium* by at least 241 bp (i.e., ~19%).

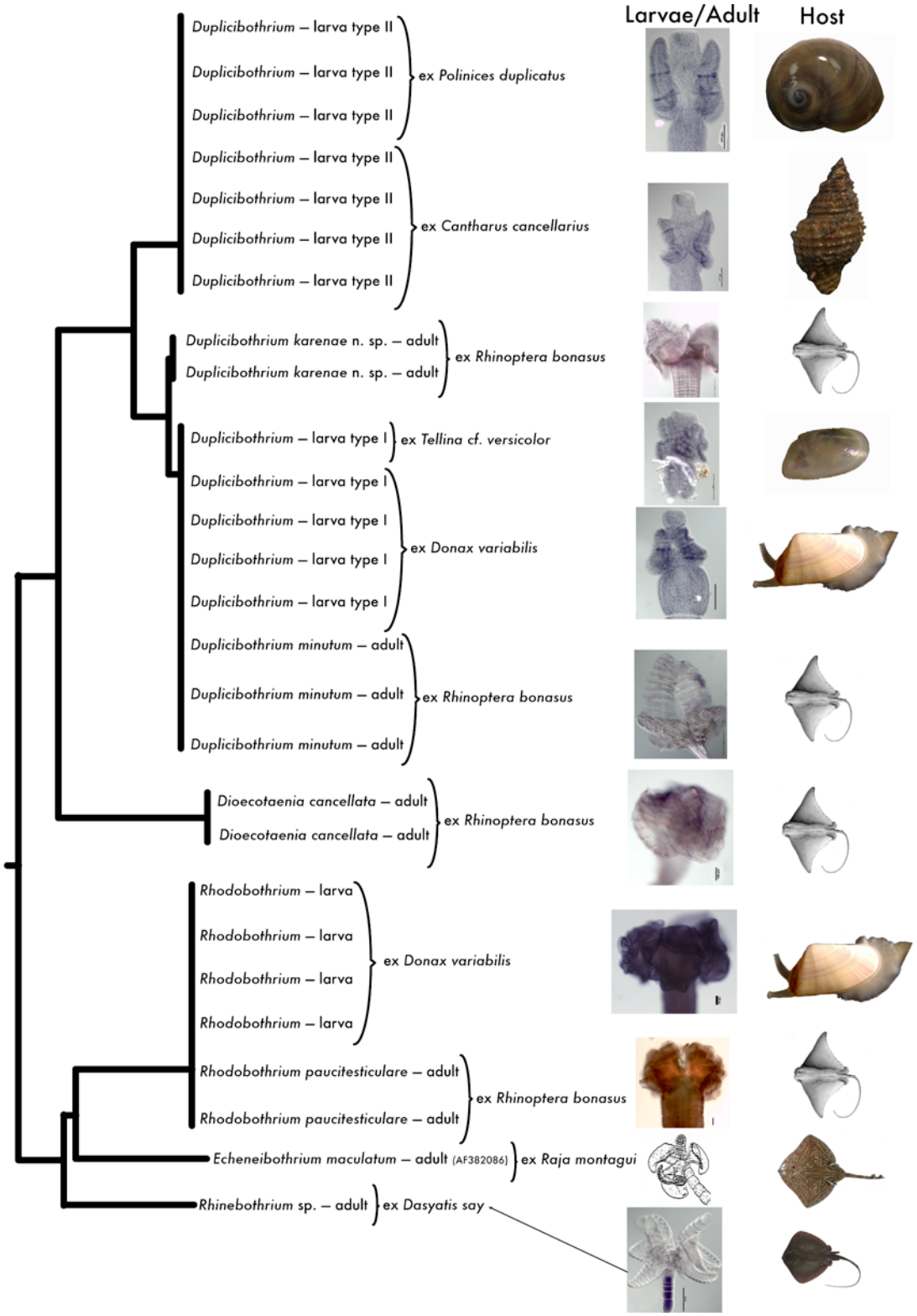

Figure 29. Neighbor-joining tree based on 1,283 base pairs of the 28S LSU (D1-D3) rDNA gene region.

	Duplicibothrium – larva type II	*Duplicibothrium karenae* n. sp. – adult	*Duplicibothrium minutum* – adult/larva type I	*Dioecotaenia cancellata* – adult	*Rhodobothrium paucitesticulare* – adult/larva type	*Echeneibothrium maculatum* – adult (AF382086)	*Rhinebothrium* sp. – adult
Duplicibothrium – larva type II	0	63 (5%)	75 (6%)	175 (14%)	250 (19%)	257 (20%)	254 (20%)
Duplicibothrium karenae n. sp. – adult	63 (5%)	0	11 (1%)	197 (15%)	241 (19%)	255 (20%)	262 (20%)
Duplicibothrium minutum – adult/larva type I	75 (6%)	11 (1%)	0	199 (15%)	254 (20%)	291 (23%)	266 (21%)
Dioecotaenia cancellata – adult	175 (14%)	197 (15%)	199 (15%)	0	266 (21%)	286 (22%)	270 (21%)
Rhodobothrium paucitesticulare – adult/larva type	250 (19%)	241 (19%	254 (20%)	266 (21%)	0	187 (15%)	193 (15%)
Echeneibothrium maculatum – adult (AF382086)	257 (20%)	255 (20%)	291(22%)	286 (22%)	187 (15%)	0	204 (16%)
Rhinebothrium sp. – adult	254 (20%)	262 (20%)	266 (21%)	270 (21%)	193 (15%)	204 (16%)	0

Table 7. Pairwise distance matrix showing overall sequence similarity of the 1,283 base pair 28S gene region.

Discussion

Size of clams and prevalence data were collected to understand possible patterns of infection in *Donax variabilis* with tapeworm larvae. While no thorough correlation statistics were applied to the size and prevalence data, general trends could be observed. Of the 405 specimens of *Donax variabilis* collected 79% were in the 14.5-20.5 mm size range; similarly, of the 79 larvae collected in July 2006, 76% were in clams between 14.5 and 20.5 mm. This suggests that the chance to encounter *Donax variabilis* infected with *Duplicibothrium minutum* is size independent. For the larvae of *R. paucitesticulare*, not enough were sampled infer a trend. It is also interesting to note that in *Donax variabilis* the prevalence of *Duplicibothrium minutum* larvae (4-17%) is much higher than that of *R. paucitesticulare* larvae (0-2%). Similarly the intensity of infection in *R. bonasus* of *Duplicibothrium minutum* (1->100) is higher than that of *R. paucitesticulare* (1-28).

This study was the first to examine the larvae of *Duplicibothrium minutum* and *R. paucitesticulare* using SEM (see Figs. 26A, 28). Unfortunately, no microthriches were observed on larvae of *Duplicibothrium minutum* examined so no comments can be made here. However, short filitriches were observed on the outer covering of the *R. paucitesticulare* larvae. Since one of the proposed functions of filitriches is absorption of nutrients (e.g., Caira and Littlewood, 2001), this suggests that the covering might allow absorption of nutrients necessary to maintain the encysted larva. The microthrix pattern observed on the scolex is similar to that of the adult. Specifically, the distal bothridial surfaces in both larva and adult are covered

with small filitriches. The lack of microtriches posterior to the scolex could be due to disruption upon manual excision. Examination of larvae of other species of *Rhodobothrium* with SEM, such as the larva of *R. mesodesmatum*, would be beneficial to confirm these observations.

Based on this study, larvae of two tapeworm species, *Duplicibothrium minutum* and *Rhodobothrium paucitesticulare*, collected from *Donax variabilis* were identified using morphology and molecular sequence data. The second group resulting from the neighbor-joining analysis consisted of *D. karenae* adults only, while a third group consisted of *Duplicibothrium* larva type II collected from two species of gastropods. Three species of *Duplicibothrium* were described from *R. bonasus* herein, two of which, *D. minutum* and *D. karenae* were adults sequenced in this analysis. It appears that the *Duplicibothrium* larva type II might represent larva of *D. mergacephalum*, adults of which were not sequenced as part of this study. This is supported by examination of the scolex morphology; these larvae possess approximately 28 rows of loculi as compared to 27-33 rows of loculi observed in adults of *D. mergacephalum*. Moreover, it is interesting to note that larvae of *D. minutum* were found to parasitize bivalves, while putatively larvae of *D. mergacephalum* were found to parasitize predaceous gastropods. Sequence similarity of *D. karenae* with *D. minutum* might suggest bivalves as candidates for intermediate hosts of *D. karenae*.

Based on the sequence data generated for species of *Duplicibothrium* and *Rhodobothrium*, no intraspecific sequence variation was observed either between

adults or multiple larvae, nor between larvae and adults. Interspecific variation in *Duplicibothrium* ranged from 11 bp (1%) between *D. karenae* and *D. minutum* to 75 bp (6%) between *D. minutum* and *Duplicibothrium* larva type II (putatively larvae of *D. mergacephalum*). Future inclusion of larvae of *D. karenae* and adults of *Duplicibothrium* larva type II (i.e., possible adults of *D. mergacephalum*), as well as data from the two species of *Duplicibothrium* (i.e., *D. cairae* and *D. paulum*) described by Ruhnke (1994) would allow a more complete investigation of intraspecific versus interspecific sequence variation in *Duplicibothrium*.

This information allows speculation on the hypothesized lifecycles of these two species. One hypothesis is a three-host lifecycle. The first intermediate host would be a copepod, which ingests an egg of either *Duplicibothrium minutum* or *R. paucitesticulare*. The second intermediate host, *Donax variabilis*, would then ingest the larva via the copepod. *Donax variabilis* is then preyed upon by the definitive host, *Rhinoptera bonasus*, which thereby ingests the larva. An alternate hypothesis is a two-host lifecycle. In this scenario, the eggs of *Duplicibothrium minutum* or *R. paucitesticulare* are directly ingested by *Donax variabilis*. Subsequently, *Donax variabilis* is ingested by the definitive host, *R. bonasus*, in which the larvae of *Duplicibothrium minutum* and *R. paucitesticulare* mature into adults. Information regarding food particle size limitation in feeding habits of *Donax variabilis* was not available to determine whether the bivalve is more likely to ingest copepods or eggs. The latter hypothesis, comprising a two-host lifecycle, albeit more unconventional, seems highly probable given the observations of *R. bonasus* frequenting the same

habitat as *Donax variabilis*, thus releasing eggs into the environment through defecation which would be available for direct ingestion by *Donax variabilis*.

Several additional factors support *R. bonasus* acquiring infections of *Duplicibothrium minutum* and *R. paucitesticulare* by consuming *Donax variabilis*. One factor is the high density of infected *Donax variabilis* (e.g., in July almost one in five specimens collected was infected with *Duplicibothrium minutum*) in the same locality as the infected cownose rays, coupled with the observation in this study of crushed coquina shells in the stomach contents of a cownose ray. Moreover, Collins et al. (2007) reported evidence that *R. bonasus* modifies its behavior to readily consume abundant and available prey, and possibly consuming *Donax variabilis* when it is most abundant (as many as 32,000 per linear yard in the summer (northern hemisphere) [Loesch, 1957]).

Some speculation can be made about the lifecycle of the *Duplicibothrium* larva type II. Interesting is the fact that *Duplicibothrium* larva type II, morphologically and molecularly distinct from *Duplicibothrium minutum* and *Duplicibothrium karenae*, were found to parasitize predaceous gastropods (see Fig. 29). In the case of these larvae, it is possible bivalves serve as first or second intermediate hosts and that predaceous gastropods could be acting as a paratenic host by feeding on infected bivalves as reported by Cake (1977) for *Fasciolaria lilium hunteria* (Perry). These intermediate hosts (bivalves) or paratenic hosts (gastropods) are then preyed upon by the definitive host.

Ultimately, experimental infections, such as exposure of uninfected *Donax variabilis* to *Duplicibothrium* and *R. paucitesticulare* eggs and feeding of infected *Donax variabilis* to naïve *R. bonasus* through feeding experiments would do much to confirm the lifecycle hypotheses put forth in this study.

Literature Cited

Al Kawari, K. S. R., M. F. A. Saoud, and M. Q. A. Wanas (1994) Helminth parasites of fishes from the Arabian Gulf 7. On *Eniochobothrium qatarense* sp. nov. (Cestoda: Lecanicephalidea) and the affinities of *Eniochobothrium* Shipley and Hornell, 1906, *Litobothrium* Dailey, 1969 and *Renyxa* Kurochkin an Slankis, 1973. *Japanese Journal of Parasitology*, 43, 97-104.

Andrews, J. (1977) *Shells and Shores of Texas*. University of Texas Press, Austin Texas, 365 pp.

Blaylock, R. A. (1989) A massive school of cownose rays, *Rhinoptera bonasus* (Rhinopteridae), in Lower Chesapeake Bay, Virginia. *Copeia*, 1989, 3, 744-748.

Blaylock, R. A. (1993) Distribution and abundance of the cownose ray, *Rhinoptera bonasus*, in Lower Chesapeake Bay. *Estuaries*, 16, 2, 255-263.

Bonsdorff, E. and W. G. Nelson (1992) The ecology of coquina clams *Donax variabilis* Say, 1822, and *Donax parvula* Philippi, 1849, on the east coast of Florida. *The Veliger*, 35, 358-365.

Brickle, R., P. D. Olson, D. T. J. Littlewood, A. Bishop, and A. I. Arkhipkin (2001) Parasites of *Loligo gahi* from waters off the Falkland Islands, with a phylogenetically

based identification of their cestode larvae. *Canadian Journal of Zoology*, 79, 2,289-2296.

Brooks, D. R. (1982) A simulations approach to discerning possible sister-groups of *Dioecotaenia* Schmidt, 1969 (Cestoda: Tetraphyllidea: Dioecotaeniidae). *Proceeding of the National Helminthological Society of Washington,* 49, 1, 56-61.

Brooks, D. R. and R. Barriga (1995) *Serendip deborahae* n. gen. and n. sp. (Eucestoda: Tetraphyllidea: Serendipidae n. fam.) in *Rhinoptera steindachneri* Evermann and Jenkins, 1891 (Chondrichthyes: Myliobatiformes: Myliobatidae) from southeastern Ecuador. *Journal of Parasitology*, 81, 1, 80-84.

Caira, J. N. and C. Healy (2004) Elasmobranchs as hosts of metazoan parasites. In: J. C. Carrier, J. A. Musick, M. R. Heithaus (eds), *Biology of Sharks and Their Relatives*. CRC Press, Boca Raton, Florida, 524-545.

Caira, J. N. and D. T. J. Littlewood (2001) Worm, Platyhelminthes. In: S. A. Levin (ed.) *Encyclopedia of Biodiversity*. Academic Press, San Diego, California, 5, 863-899.

Caira, J. N., K. Jensen, C. J. Healy (1999) On the phylogenetic relationships among tetraphyllidean, lecanicephalidean, and diphyllidean tapeworm genera. *Systematic Parasitology*, 42, 77-151.

Cake, E. W. Jr. (1975) Larval and postlarval cestode parasites of shallow water, benthic mollusks of the Gulf of Mexico from the Florida Keys to the Mississippi Sound. *Dissertation Abstracts International*. 36, 1, 40-41B.

Cake, E. W. Jr. (1976) A key to larval cestode of shallow-water, benthic mollusks of the northern Gulf of Mexico. *Proceedings of the Helminthological Society of Washingon,* 43, 160-171.

Cake, E. W. Jr. (1977) Experimental infection studies with bothrido-plerocerocoids of *Rhinebothrium* sp. (Cestoda, Tetraphyllidea) and two intermediate molluscan hosts. *Northeast Gulf Science,* 1, 55-59.

Cake, E. W. Jr. (1978) Larval cestode parasites of edible mollusks of the northeastern Gulf of Mexico. *Gulf Research Reports,* 6, 1, 1-8.

Cake, E. W. Jr. and R. W. Menzel (1980) Infections of *Tylocephalum* metacestodes in commercial oysters in commercial oysters and three predaceous gastropods of the eastern Gulf of Mexico. *Proceedings of National Shellfish Association,* 70, 94-104.

Campbell, R. A. (1969) New species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from Chesapeake Bay, Virginia. *Journal of Parasitology*, 55, 3, 559-570.

Campbell, R. A. (1975) Tetraphyllidean cestodes from western north Atlantic selachians with descriptions of two new species. *Journal of Parasitology*, 61, 2, 265-270.

Campbell, R. A. and J. Carvajal (1975) A revision of some trypanorhynchs from the North Atlantic described by Edwin Linton. *Journal of Parasitology*, 61, 1,016-1,022.

Campbell, R. A. and J. G. Carvajal (1979) Synonymy of the phyllobothriid genera *Rhodobothriumi* Linton, 1889, *Inermiphyllidium* Riser, 1955, and *Sphaerobothrium* Euzet, 1959 (Cestoda: Tetraphyllidea). *Proceedings of the Helminthological Society of Washington*, 46, 1, 88-97.

Campbell, R. A. and A. D. Williams (1984) *Tylocephalum* Linton, 1890 (Cestoda: Lecanicephalidea) from the cownose ray, *Rhinoptera bonasus* (Mitchill, 1815) with a discussion of its validity and systematic relationships. *Proceedings of the Helminthological Society of Washington*, 51, 121-134.

Carvajal, J. and R. A. Campbell (1975) *Rhinoptericola megacantha* gen. et sp. n., representing a new family of trypanorhynch cestodes from the cownose ray, *Rhinoptera bonasus* (Mitchill 1815). *Journal of Parasitology*, 61, 1023-1030.

Cheng, T. C. (1966) The coracidium of the cestode *Tylocephalum* and the migration and fate of this parasite in the American oyster, *Crassostrea virginica*. *Transactions of the American Microscopical Society*, 85, 2, 246-255.

Collins, A. B., M. R. Heupel, R. E. Heuter, and P. J. Motta (2007) Hard prey specialist or opportunistic generalists? An examination of the diet of the cownose ray, *Rhinoptera bonasus*. *Marine and Freshwater Research*, 58, 135-144.

Compagno, L. J. V. (1999) Checklist of Living Elasmobranchs. In: W. C. Hamlett (ed) *Sharks, Skates, and Rays*. Johns Hopkins University Press, Baltimore, Maryland, 471-498.

Euzet, L. (1994) Order Tetraphyllidea Carus, 1863. In: Khalil, L. F., A. Jones, R. A. Bray (eds), *Keys to the Cestode Parasites of Vertebrates*. CAB International, Wallingford, England, 149-194.

Healy, C. J. (2006) A revision of selected Tetraphyllidea (Cestoda): *Caulobothrium, Rhabdotobothrium, Rhinebothrium, Scalithrium,* and *Spongiobothrium*. Unpublished Ph. D. dissertation, University of Connecticut, Storrs, Connecticut, 361+pp.

Hutton, R. F. (1964) A second list of parasites and coastal animals of Florida. *Transactions of the American Microscopical Society*, 83, 439-447.

Ivanov, V. A. and R. A. Campbell (2000) Emendation of the generic diagnosis of *Tylocephalum* (Cestoda: Lecanicephalidea: Teteragonocephalidae), and description of *Tylocephalum brooksi* n. sp. *Journal of Parasitology*, 86, 1085-1092.

Jensen, K. (2005) Tapeworms of the elasmobranchs (Part 1). A monograph on the Lecanicephalidea (Platyhelminthes, Cestoda). *Bulletin of the University of Nebraska State Museum*, 18, ii-241.

Jones, D. S., I. R. Quitmeyer, and C. F. T. Andrus (2004) Seasonal shell growth and longevity in *Donax variablis* from Northeastern Florida: evidence from oxygen isotopes. *Journal of Shellfisheries Research*, 23, 3, 704-714.

Lauckner, G. (1983) Diseases of Mollusca. In: Kinne O. (ed) *Diseases of Marine Mammals*. Biologische Anstalt Helgoland, Hamburg, Germany, 762-783.

Linton, E. (1890) Notes on Entozoa of marine fishes of New England, with descriptions of several new species. Part II. *Report of the United States Commisioner of Fisheries* (1887), Washington D.C., 15, 718-899.

Linton, E. (1897) Notes on cestode parasites of fishes. *Proceedings of the United States National Museum*, 20, 423-456.

Linton, E. (1901) Parasites of fishes of the Wood Hole region. *Bulletin of the United States Fish Commission* (1899), 19, 405-492.

Littlewood, D. T. J., M. Curini-Galletti, and E. A. Herniou (2000) The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution*, 16, 449-466.

Loesch, H. (1957) Studies of the ecology of two species of *Donax* on Mustand Island, Texas. *Publications of the Institute of Marine Science*, 4, 2, 201-227.

Mattis, T. E. (1986) Development of two tetrarhychidian cestodes from the northern Gulf of Mexico. Unpublished Ph. D. dissertation, University of Southern Mississippi, Hattiesburg, Mississippi, 177 pp.

Mariaux J. and P. D. Olson (2001) Cestode systematics in the molecular era. In: D. T. J. Littlewood, R. A. Bray (eds) *Interrelationships of the Platyhelminthes*. Taylor and Francis, New York, New York, 127-134.

Mayes, M. A. and D. R. Brooks (1981) Cestode parasites of some Venezuelan stingrays. *Proceedings of the Biological Society of Washington*, 93, 1230-1238.

McEachran, J. D. and B. Seret (1990) Rhinopteridae. *Checklist of the Eastern Tropical Atlantic*. 71-72.

Mikkelsen, P. S. (1985) A comparison of two Florida populations of the coquina clam, *Donax variabilis* Say, 1822 (Bivalvia: Donacidae). II. Growth rates. *The Veliger*, 27, 308-311.

Moravec, F., V. M. Vidal-Martinez, J. Vargas-Vazquez, C. Vivas-Rodrduez, D. Gonzalez-Solis, E. Mendoza-Franco, R. Sima-Alvarez, and J. Guemez-Ricalde (1997) Helminth parasites of *Epinephelus morio* (Pisces: Serranidae) of the Yucatan Peninsula, southeastern Mexico. *Folia Parasitologica*, 44, 255-266.

Morrison, J. P. E. (1971) Western Atlantic *Donax*. *Proceedings of the Biological Society of Washington*, 83, 545-568.

Mudry, D. R. and M. D. Dailey (1971) Postembryonic development of certain tetraphyllidean and trypanorhynchan cestodes with a possible alternate life cycle for the order Trypanorhyncha. *Canadian Journal of Zoology*, 49, 1,249-1253.

Multiple Sequence Alignment by ClustalW, Online. Internet. Kyoto University Bioinformatics Center. 20 July 2006. < http://align.genome.jp/>.

Neer, J. A. and B. A. Thompson (2005) Life history of the cownose ray, *Rhinoptera bonasus*, in the northern Gulf of Mexico, with comments on geographic variability in life history traits. *Environmental Biology of Fishes*, 73, 321-331.

Nelson, J. S., E. J. Crossman, H. Espinosa-Perez, L. T. Findley, C. R. Gilbert, R. N. Lea, and J. D. Williams (2004) Common and scientific names of fishes from the United

States, Canada, and Mexico. American Fisheries Society: Bethesda, Maryland, Special Publication 29, vii-386.

Olson, P. D., D. T. J. Littlewood, R. A. Bray, and J. Mariaux (2001) Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution*, 19, 3, 443-467.

Olson, P. D., T. R. Ruhnke, J. Sanney and T. Hudson (1999) Evidence of host specific clades of tetraphyllidean tapeworms (Platyhelminthes: Eucestoda) revealed by analysis of 18ssrDNA. *International Journal for Parasitology*, 29, 1,465-1,476.

Palm, H. W. (1999) *Nybelinia* Poche, 1926, *Heteronybelina* gen. nov. and *Mixonybelinia* gen. nov. (Cestoda, Trypanorhyncha) in the collections of The Natural History Museum, London. *Bulletin of The Natural History Museum London (Zoology)*, 65, 2, 133-153.

Palm, H.W. (2004) *The Trypanorhyncha Diesing, 1863*. PKSPL-IPB Press, Bogor, x + 710.

Quitmeyer, I. R., D. S. Jones, and C. F. T. Andrus (2004) Seasonal collection of coquina clams (*Donax variabilis* Say, 1822) during the Archaic and St. Johns periods in coastal northeast Florida. In: D. Bar-Yosef (ed.), *Archaeomalacology: molluscs in former environments of human behavior*. Oxbow Press, London, 18-28.

Reyda, F. B. and P. D. Olson (2003) Cestodes of cestodes of Peruvian freshwater stingrays. *Journal of Parasitology*, 89, 1,018-2,024.

Ride, W. D. L., H. G. Cogger, C. Dupuis, O. Kraus, A. Minella, F. C. Thompson, and P. K. Tubbs (1999) *International Code of Zoological Nomenclature*. 4th Edition. The International Trust for Zoological Nomenclature, The Natural History Museum, London, 306 pp.

Riser, N. (1956) Early larval stages of two cestodes from elasmobranch fishes. *Proceedings of the Helminthological Society of Washington*, 23, 2, 120-124.

Rogers, C., C. Roden, R. Lohoefener, and K. Mullin (1990) Behavior, distribution, and relative abundance of cownose ray schools *Rhinoptera bonasus* in the northern Gulf of Mexico. *Northeast Gulf Science*, 11, 1, 69-76.

Ruhnke, T. R. (1994) Resurrection of *Anthocephalum* Linton, 1890 (Cestoda: Tetraphyllidea) and taxonomic information on five proposed members. *Systematic Parasitology*, 29, 159-176.

Ruhnke, T. R., S. S. Curran, and T. Holbert (2000) Two new species of *Duplicibothrium* Williams & Campbell, 1978 (Tetraphyllidea: Serendipidae) from the Pacific cownose ray *Rhinoptera steindachneri*. *Systematic Parasitology*, 47, 135-143.

Ruppert, E. E. and R. S. Fox (1988) *Seashore Animals of the Southeast*. University of South Carolina Press, Columbia, South Carolina, 377 pp.

Schmidt, G. D. (1969) *Dioecotaenia cancellata* (Linton 1890) gen. et comb. n., a dioecious cestode (Tetraphyllidea) from the cownose ray, *Rhinoptera bonasus* (Mitchell), in Chesapeake Bay, with the proposal of a new family, Dioecotaeniidae. *Journal of Parasitology*, 55, 271-275.

Schwartz, F. J. (1965) Inter-American migrations and systematics of the Western Atlantic cownose ray, *Rhinoptera bonasus*. (Abstract). Meeting of the Association of Island Marine Laboratories of the Caribbean, 6, 1.

Schwartz, F. J. (1990) Mass migratory congregation and movements of several species of cownose rays, genus *Rhinoptera:* A worldwide review. *The Journal of the Elisha Mitchell Scientific Society*, 106, 1, 10-13.

Shipley, A. E. and J. Hornell (1906) Report on the cestode and nematode parasites from the marine fishes of Ceylon. Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar (Herdman), Part 5, 43-96.

Smith, J. W. and J. V. Merriner (1985) Food habits and feeding behavior of the cownose ray, *Rhinoptera bonasus*, in Lower Chesapeake Bay. *Estuaries*, 8, 3, 305-310.

Smith, J. W. and J. V. Merriner (1987) Age and growth, movements and distribution of the cownose ray, *Rhinoptera bonasus*, in Chesapeake Bay. *Estuaries*, 10, 2, 153-164.

Southwell, T. (1924) The pearl-inducing worm in the Ceylon pearl oyster. *Annals of Tropical Medicine and Parasitology*, 18, 37-53.

Tyler, G. A. (2006) Tapeworms of the elasmobranchs (Part 2). A monograph on the Diphyllidea (Platyhelminthes, Cestoda). *Bulletin of the University of Nebraska State Museum*, 20, ii-142.

Tyler, G. A. and J. N. Caira (1999) Two new species of *Echinobothrium* (Cestoidea: Diphyllidea) from myliobatiform elasmobranches in the Gulf of California, Mexico. *Journal of Parasitology*, 85, 2, 327-335.

Wardle, W. J. (1974) A survey of the occurrence, distribution and incidence of infection of helminth parasites of marine and estuarine Mollusca from Galveston, Texas. Unpublished Ph. D. Dissertation. Texas A&M University, College Station, Texas, 322 pp.

Williams, A. D. and R. A. Campbell (1977) A new tetraphyllidean cestode, *Glyphobothrium zwerneri* gen. et sp. n., from the cownose ray, *Rhinoptera bonasus* (Mitchill 1815). *Journal of Parasitology*, 63, 775-779.

Williams, A. D. and R. A. Campbell (1978) *Duplicibothrium minutum* gen. et sp. n. (Cestoda: Tetraphyllidea) from the cownose ray, *Rhinoptera bonasus* (Mitchill 1815). *Journal of Parasitology*, 64, 835-837.

Williams, A. D. and R. A. Campbell (1980) *Echinobothrium bonasum* sp. n., a new cestode from the cownose ray in the western north Atlantic. *Journal of Parasitology*, 66, 1,036-1,038.

Wilson, J. G. (1999) Population dynamics and energy budget for a population of *Donax variabilis* (Say) on an exposed South Carolina beach. *Journal of Experimental Marine Biology*, 239, 61-83.

Appendix 1

Aligned sequences for the LSU D1-D3 region of the (28S) rDNA gene

```
           1         10         20         30         40         50         60
Dioeco_ca  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAGAGCCCAGCACCGAAGCC
Dioeco_ca  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAGAGCCCAGCACCGAAGCC
Duplici_I  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_mi  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_mi  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_mi  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplici_I  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplici_I  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplici_I  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplici_I  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_ka  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_ka  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_II  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_II  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_II  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_II  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_II  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_II  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Duplic_II  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAGCACCGAAGCC
Rhodo_pau  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAATAGCCCAGCACCGAAGCC
Rhodo_pau  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAATAGCCCAGCACCGAAGCC
Rhodo_lar  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAATAGCCCAGCACCGAAGCC
Rhodo_lar  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAATAGCCCAGCACCGAAGCC
Rhodo_lar  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAATAGCCCAGCACCGAAGCC
Rhodo_lar  --CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAATAGCCCAGCACCGAAGCC
Rhine_sp   ---------------------------------GAAGAGGGAATAGCCCAGCACCGAAGCC
Echen_ma   AACTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAGAAGCCCAGCACCGAAGCC

           61        70         80         90         100        110
Dioeco_ca  TGCGGCAGGTTTGCTGTTAGGCAATGTGGTGTTTGGGTCGACTCGCGGGATTGCCACTCC
Dioeco_ca  TGCGGCAGGTTTGCTGTTAGGCAATGTGGTGTTTGGGTCGACTCGCGGGATTGCCACTCC
Duplici_I  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplic_mi  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplic_mi  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplic_mi  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplici_I  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplici_I  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplici_I  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGNGATTGCCGCTCC
Duplici_I  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplic_ka  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplic_ka  TGTGGCTATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGATTCGTGGGATTGCCGCTCC
Duplic_II  TGTGGCCATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGACTCGCGGGACTGCCGCTCC
Duplic_II  TGTGGCCATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGACTCGCGGGACTGCCGCTCC
Duplic_II  TGTGGCCATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGACTCGCGGGACTGCCGCTCC
Duplic_II  TGTGGCCATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGACTCGCGGGACTGCCGCTCC
Duplic_II  TGTGGCCATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGACTCGCGGGACTGCCGCTCC
Duplic_II  TGTGGCCATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGACTCGCGGGACTGCCGCTCC
Duplic_II  TGTGGCCATTTGGCTACTAGGCAATGTGGTGTTTAGGCCGACTCGCGGGACTGCCGCTCC
Rhodo_pau  TGCGGTAGTTTTACTGTTAGGCAATGTGGTGTTTGGGTCGGCTCGCGGGGTTGCCACTCC
Rhodo_pau  TGCGGTAGTTTTACTGTTAGGCAATGTGGTGTTTGGGTCGGCTCGCGGGGTTGCCACTCC
Rhodo_lar  TGCGGTAGTTTTACTGTTAGGCAATGTGGTGTTTGGGTCGGCTCGCGGGGTTGCCACTCC
Rhodo_lar  TGCGGTAGTTTTACTGTTAGGCAATGTGGTGTTTGGGTCGGCTCGCGGGGTTGCCACTCC
Rhodo_lar  TGCGGTAGTTTTACTGTTAGGCAATGTGGTGTTTGGGTCGGCTCGCGGGGTTGCCACTCC
Rhodo_lar  TGCGGTAGTTTTACTGTTAGGCAATGTGGTGTTTGGGTCGGCTCGCGGGGTTGCCACTCC
Rhine_sp   TGTGGCAGTTTTGCTGCTAGGCAATGTGGTGTTTGGGTCGGCTCGCGGAATTGCCACTCC
Echen_ma   TGCGGCAGTTTTGCTGTTAGGCAATGTGGTGTTTGGGTCGGTTCGTGGGATTGCCACTCC
```

```
           120        130        140        150        160        170
Dioeco_ca  ACTCGAAGTCCAGCATTGAGTATGGT-TACTGGACTCGGCCCAGAGAGGGTGATAGGCCC
Dioeco_ca  ACTCGAAGTCCAGCATTGAGTATGGT-TACTGGACTCGGCCCAGAGAGGGTGATAGGCCC
Duplici_I  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_mi  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_mi  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_mi  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplici_I  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplici_I  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplici_I  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplici_I  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_ka  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_ka  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_II  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_II  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_II  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_II  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_II  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Duplic_II  ACTCGAAGTCCAGCATTGAGTATGGT-CACTGGATTTGGCCCAGAGAGGGTGATAGGCCC
Rhodo_pau  ACTCGAAGTCCAGCATTGAGTATGGTCCACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC
Rhodo_pau  ACTCGAAGTCCAGCATTGAGTATGGTCCACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC
Rhodo_lar  ACTCGAAGTCCAGCATTGAGTATGGTCCACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC
Rhodo_lar  ACTCGAAGTCCAGCATTGAGTATGGTCCACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC
Rhodo_lar  ACTCGAAGTCCAGCATTGAGTATGGTCCACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC
Rhodo_lar  ACTCGAAGTCCAGCATTGAGTATGGTCCACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC
Rhine_sp   ACTCGAAGTCCAGCATTGAGTATGGTCCACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC
Echen_ma   ACTCGAAGTCCAGCATTGAGTATGGTCTACTGGATTTGGCCCAGAGAGGGTGAAAGGCCC

           180        190        200        210        220        230
Dioeco_ca  GTACGGGTGGAGGC-TCAGACAAGCGAGGCAATTCACCAGGTCGACCTTAGAGTCGGGTT
Dioeco_ca  GTACGGGTGGAGGC-TCAGACAAGCGAGGCAATTCACCAGGTCGACCTTAGAGTCGGGTT
Duplici_I  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_mi  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_mi  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_mi  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplici_I  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplici_I  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplici_I  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplici_I  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_ka  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_ka  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_II  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_II  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_II  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_II  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_II  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Duplic_II  GTACGGGTGGAGTT-TCAGACATGTAAGGCAGTTCACCAGGTCGACCTTCGAGTCGGGTT
Rhodo_pau  GTACGGGTGGAGGTTTCAGACAAGTAAGGCAACTCACTAGGCCGGCCTTAGAGTCGGGTT
Rhodo_pau  GTACGGGTGGAGGTTTCAGACAAGTAAGGCAACTCACTAGGCCGGCCTTAGAGTCGGGTT
Rhodo_lar  GTACGGGTGGAGGTTTCAGACAAGTAAGGCAACTCACTAGGCCGGCCTTAGAGTCGGGTT
Rhodo_lar  GTACGGGTGGAGGTTTCAGACAAGTAAGGCAACTCACTAGGCCGGCCTTAGAGTCGGGTT
Rhodo_lar  GTACGGGTGGAGGTTTCAGACAAGTAAGGCAACTCACTAGGCCGGCCTTAGAGTCGGGTT
Rhodo_lar  GTACGGGTGGAGGTTTCAGACAAGTAAGGCAACTCACTAGGCCGGCCTTAGAGTCGGGTT
Rhine_sp   GTACGGGTGGAGGTGTCAGACAAGTGAGGCAGTTCACTAGGCCGGCCTTAGAGTCGGGTT
Echen_ma   GTACGGGTGGAGGTATCAGACATGTGAGGCAATTCACCAGACCGGCCTTAGAGTCGGGTT
```

```
           240       250       260       270       280       290
Dioeco_ca  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACTAACACGA
Dioeco_ca  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACTAACACGA
Duplici_I  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_mi  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_mi  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_mi  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplici_I  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplici_I  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplici_I  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplici_I  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_ka  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_ka  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_II  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_II  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_II  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_II  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_II  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Duplic_II  GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACAAGCACGA
Rhodo_pau  GTTTGGGAATGCAGCCCAAAGAGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA
Rhodo_pau  GTTTGGGAATGCAGCCCAAAGAGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA
Rhodo_lar  GTTTGGGAATGCAGCCCAAAGAGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA
Rhodo_lar  GTTTGGGAATGCAGCCCAAAGAGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA
Rhodo_lar  GTTTGGGAATGCAGCCCAAAGAGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA
Rhodo_lar  GTTTGGGAATGCAGCCCAAAGAGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA
Rhine_sp   GTTTGAGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA
Echen_ma   GTTTGAGAATGCAGCCCAAAGCGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGA

           300       310       320       330       340       350
Dioeco_ca  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Dioeco_ca  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplici_I  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_mi  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_mi  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_mi  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplici_I  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplici_I  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplici_I  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplici_I  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_ka  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_ka  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_II  GTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_II  GTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_II  GTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_II  GTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_II  GTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Duplic_II  GTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Rhodo_pau  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Rhodo_pau  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Rhodo_lar  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Rhodo_lar  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Rhodo_lar  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Rhodo_lar  GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Rhine_sp   GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
Echen_ma   GTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGTAAA
```

```
Dioeco_ca  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCTAGCCTGGAGAAATC
Dioeco_ca  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCTAGCCTGGAGAAATC
Duplici_I  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_mi  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_mi  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_mi  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplici_I  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplici_I  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplici_I  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplici_I  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_ka  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_ka  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_II  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_II  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_II  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_II  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_II  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Duplic_II  CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAATCCTGGAGAATTC
Rhodo_pau  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC
Rhodo_pau  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC
Rhodo_lar  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC
Rhodo_lar  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC
Rhodo_lar  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC
Rhodo_lar  CAGTACGTGAAGCCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC
Rhine_sp   CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC
Echen_ma   CAGTACGTGAAACCGCATGCAGGTAAACGGGTGGCGTCAAGCTGCAAGCCCAGAGGATTC

           420        430        440        450        460        470
Dioeco_ca  AGCTGGCTAGGATGTTGTAA--GCGCCTGTCGCTTCG-CCAGTCAGGGTTAGATGTGGTG
Dioeco_ca  AGCTGGCTAGGATGTTGTAA--GCGCCTGTCGCTTCG-CCAGTCAGGGTTAGATGTGGTG
Duplici_I  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplic_mi  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplic_mi  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplic_mi  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplici_I  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplici_I  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplici_I  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplici_I  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplic_ka  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplic_ka  AGCCGGCTAGTGTGTCGTGAATGCGCCTG-TGCATCA-TCAGTCGGTGTAAGATGTGATA
Duplic_II  AGCCGGCTAGTGTGTCGTATATGCGCCTGGCGCTTCA-TCAGTCAGAATAAGATGTGATA
Duplic_II  AGCCGGCTAGTGTGTCGTATATGCGCCTGGCGCTTCA-TCAGTCAGAATAAGATGTGATA
Duplic_II  AGCCGGCTAGTGTGTCGTATATGCGCCTGGCGCTTCA-TCAGTCAGAATAAGATGTGATA
Duplic_II  AGCCGGCTAGTGTGTCGTATATGCGCCTGGCGCTTCA-TCAGTCAGAATAAGATGTGATA
Duplic_II  AGCCGGCTAGTGTGTCGTATATGCGCCTGGCGCTTCA-TCAGTCAGAATAAGATGTGATA
Duplic_II  AGCCGGCTAGTGTGTCGTATATGCGCCTGGCGCTTCA-TCAGTCAGAATAAGATGTGATA
Duplic_II  AGCCGGCTAGTGTGTCGTATATGCGCCTGGCGCTTCA-TCAGTCAGAATAAGATGTGATA
Rhodo_pau  AGCCGGTTAGAGTGTAGT--ACGCGCTTG-CGTGTCAAACGGCCAGTGTAAGATTGGGTA
Rhodo_pau  AGCCGGTTAGAGTGTAGT--ACGCGCTTG-CGTGTCAAACGGCCAGTGTAAGATTGGGTA
Rhodo_lar  AGCCGGTTAGAGTGTAGT--ACGCGCTTG-CGTGTCAAACGGCCAGTGTAAGATTGGGTA
Rhodo_lar  AGCCGGTTAGAGTGTAGT--ACGCGCTTG-CGTGTCAAACGGCCAGTGTAAGATTGGGTA
Rhodo_lar  AGCCGGTTAGAGTGTAGT--ACGCGCTTG-CGTGTCAAACGGCCAGTGTAAGATTGGGTA
Rhodo_lar  AGCCGGTTAGAGTGTAGT--ACGCGCTTG-CGTGTCAAACGGCCAGTGTAAGATTGGGTA
Rhine_sp   AGTCGGTTAGGGTGTAGTGTATGCGCCTGTCGCATCAACAAGTCGGAATAGGATTGGACA
Echen_ma   AACCGGCTAGAGTGTAGTGTATGCGCCTG-CGCATCGAATGGCCAGTGTAGGATTGGGTA
```

```
           480       490       500       510       520       530
Dioeco_ca  GTCTACCGTAAAACGGTGGGT-CAGGCCGTAAGGTCTGAACATGCGTACCGGGCGGAAGC
Dioeco_ca  GTCTACCGTAAAACGGTGGGT-CAGGCCGTAAGGTCTGAACATGCGTACCGGGCGGAAGC
Duplici_I  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplic_mi  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplic_mi  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplic_mi  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplici_I  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplici_I  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplici_I  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplici_I  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplic_ka  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplic_ka  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGTTCGGTTATGTGTACCGGGTGGAAGC
Duplic_II  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGCTCGGTTATGTGTACCGGGCGGAAGC
Duplic_II  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGCTCGGTTATGTGTACCGGGCGGAAGC
Duplic_II  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGCTCGGTTATGTGTACCGGGCGGAAGC
Duplic_II  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGCTCGGTTATGTGTACCGGGCGGAAGC
Duplic_II  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGCTCGGTTATGTGTACCGGGCGGAAGC
Duplic_II  GTCTACCGGGCAACGGTAGGC-CGGGCCGCAAGGCTCGGTTATGTGTACCGGGCGGAAGC
Rhodo_pau  GTCCACTGGAAGACAGTGGGC-CCTGCCGCAAGGTGTGGGTGTGTGTACCGAGTGGAAGT
Rhodo_pau  GTCCACTGGAAGACAGTGGGC-CCTGCCGCAAGGTGTGGGTGTGTGTACCGAGTGGAAGT
Rhodo_lar  GTCCACTGGAAGACAGTGGGC-CCTGCCGCAAGGTGTGGGTGTGTGTACCGAGTGGAAGT
Rhodo_lar  GTCCACTGGAAGACAGTGGGC-CCTGCCGCAAGGTGTGGGTGTGTGTACCGAGTGGAAGT
Rhodo_lar  GTCCACTGGAAGACAGTGGGC-CCTGCCGCAAGGTGTGGGTGTGTGTACCGAGTGGAAGT
Rhodo_lar  GTCCACTGGAAGACAGTGGGC-CCTGCCGCAAGGTGTGGGTGTGTGTACCGAGTGGAAGT
Rhine_sp   GTCTGCTGGAAGACAGCGGGTGCCTGCCGCAAGGTGGGTATGTGTGTACCGGGTGGTTGC
Echen_ma   GTCTACTGGAAGACAGTGGGC-CCTGCCGCAAGGTGGGGATGTGTGTACCGGGCGGAGGC

           540       550       560       570       580       590
Dioeco_ca  TGGAGCATGCCATCCATCTGTTGGACTGTCTGGCCAGTGCACTTTCTCCATGGTGAACAC
Dioeco_ca  TGGAGCATGCCATCCATCTGTTGGACTGTCTGGCCAGTGCACTTTCTCCATGGTGAACAC
Duplici_I  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_mi  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_mi  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_mi  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplici_I  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplici_I  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplici_I  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplici_I  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_ka  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_ka  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_II  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_II  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_II  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_II  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_II  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Duplic_II  CGGAGCATGCTATTCATCTGTTGGACTGGCTAGCCGGTGCACTTTCTCTATGGTGAACAC
Rhodo_pau  CGGAGCACGCTATCCGTCTGTTGGGCCATCTAGCCGGTGCACTTTCTCTGTGGTGAACAC
Rhodo_pau  CGGAGCACGCTATCCGTCTGTTGGGCCATCTAGCCGGTGCACTTTCTCTGTGGTGAACAC
Rhodo_lar  CGGAGCACGCTATCCGTCTGTTGGGCCATCTAGCCGGTGCACTTTCTCTGTGGTGAACAC
Rhodo_lar  CGGAGCACGCTATCCGTCTGTTGGGCCATCTAGCCGGTGCACTTTCTCTGTGGTGAACAC
Rhodo_lar  CGGAGCACGCTATCCGTCTGTTGGGCCATCTAGCCGGTGCACTTTCTCTGTGGTGAACAC
Rhodo_lar  CGGAGCACGCTATCCGTCTGTTGGGCCATCTAGCCGGTGCACTTTCTCTGTGGTGAACAC
Rhine_sp   CGGAGCACGCTGTTCGTCTGTTGGACTGCCTAGCCGGTGCACTTTCTCTGTGGTGGACAC
Echen_ma   CGGAACACGCTATCCGTCTATTGGGCTGCCTAGCCGGTGCACTTTCTCTGTGGTGAACAC
```

```
           600       610       620       630       640       650
Dioeco_ca  CACGATCGGTGGTATTGCCAGTCTGCTGTGGCCGAGTTGCGCTAGGTGAAT-CTCAGG-G
Dioeco_ca  CACGATCGGTGGTATTGCCAGTCTGCTGTGGCCGAGTTGCGCTAGGTGAAT-CTCAGG-G
Duplici_I  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplic_mi  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplic_mi  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplic_mi  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplici_I  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplici_I  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplici_I  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplici_I  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplic_ka  CACGATCGGTGGAATTGCCAGTCTGCNGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplic_ka  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTATTCGATGGGTTCTTGTA-A
Duplic_II  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTAGCTGATAAGTTCTTGCA-A
Duplic_II  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTAGCTGATAAGTTCTTGCA-A
Duplic_II  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTAGCTGATAAGTTCTTGCA-A
Duplic_II  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTAGCTGATAAGTTCTTGCA-A
Duplic_II  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTAGCTGATAAGTTCTTGCA-A
Duplic_II  CACGATCGGTGGAATTGCCAGTCTGCTGTAGTCAAGTCGTAGCTGATAAGTTCTTGCA-A
Rhodo_pau  CACGACCGGTGGAACTGCCAGTCTGCTATGGTCAAGTCGTGTTTGGTCGGTCCTTGTG-G
Rhodo_pau  CACGACCGGTGGAACTGCCAGTCTGCTATGGTCAAGTCGTGTTTGGTCGGTCCTTGTG-G
Rhodo_lar  CACGACCGGTGGAACTGCCAGTCTGCTATGGTCAAGTCGTGTTTGGTCGGTCCTTGTG-G
Rhodo_lar  CACGACCGGTGGAACTGCCAGTCTGCTATGGTCAAGTCGTGTTTGGTCGGTCCTTGTG-G
Rhodo_lar  CACGACCGGTGGAACTGCCAGTCTGCTATGGTCAAGTCGTGTTTGGTCGGTCCTTGTG-G
Rhodo_lar  CACGACCGGTGGAACTGCCAGTCTGCTATGGTCAAGTCGTGTTTGGTCGGTCCTTGTG-G
Rhine_sp   CACGACCGATGGTACTGTCAGTCTGCTGTGGTCAAGTCGTGTTTGGTTGGTGCTAACGTG
Echen_ma   CACGACCGGTGGTACTGCCAGTCTGTCTTAGTCAAGTCGTAGT-GGGCGGGCTTTCGG-G

           660       670       680       690       700       710
Dioeco_ca  TTCGCTGAGTGTGATCACGGGCAATTTCAGTAAACGGCGTACG-GGTGCCGCTTTGGTGG
Dioeco_ca  TTCGCTGAGTGTGATCACGGGCAATTTCAGTAAACGGCGTACG-GGTGCCGCTTTGGTGG
Duplici_I  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplic_mi  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplic_mi  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplic_mi  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplici_I  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplici_I  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplici_I  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplici_I  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------CA
Duplic_ka  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------GG
Duplic_ka  TTCATTGGGTGCGATCACAGGCAATGTCAGTACACGGCGTAGA-GGTGTCTC------GG
Duplic_II  TTTATTGGTTGCGATCACAGGCAATGTCAGTATACGGCGTAGA-GGTGTTTT------GT
Duplic_II  TTTATTGGTTGCGATCACAGGCAATGTCAGTATACGGCGTAGA-GGTGTTTT------GT
Duplic_II  TTTATTGGTTGCGATCACAGGCAATGTCAGTATACGGCGTAGA-GGTGTTTT------GT
Duplic_II  TTTATTGGTTGCGATCACAGGCAATGTCAGTATACGGCGTAGA-GGTGTTTT------GT
Duplic_II  TTTATTGGTTGCGATCACAGGCAATGTCAGTATACGGCGTAGA-GGTGTTTT------GT
Duplic_II  TTTATTGGTTGCGATCACAGGCAATGTCAGTATACGGCGTAGA-GGTGTTTT------GT
Rhodo_pau  CCTTCTGAATGCGATCACGGGCAAATTCGGTGAACGGCGTAGA-TGTGCTCTCG----GG
Rhodo_pau  CCTTCTGAATGCGATCACGGGCAAATTCGGTGAACGGCGTAGA-TGTGCTCTCG----GG
Rhodo_lar  CCTTCTGAATGCGATCACGGGCAAATTCGGTGAACGGCGTAGA-TGTGCTCTCG----GG
Rhodo_lar  CCTTCTGAATGCGATCACGGGCAAATTCGGTGAACGGCGTAGA-TGTGCTCTCG----GG
Rhodo_lar  CCTTCTGAATGCGATCACGGGCAAATTCGGTGAACGGCGTAGA-TGTGCTCTCG----GG
Rhodo_lar  CCTTCTGAATGCGATCACGGGCAAATTCGGTGAACGGCGTAGA-TGTGCTCTCG----GG
Rhine_sp   CCTACTGGATGCGATCACGGGCAATGTTGGTAGACGGCGTGAGCTTCATCTTTCA---GG
Echen_ma   TCTGCCTGCTGTGATCACGGGCGTACTTGGTATACGGCGTTAG-TGCATCTTTAC---GG
```

```
           720       730       740       750       760       770
Dioeco_ca  TGTCCGTGCGTGTTATCGGCTACTGGTTGTC--GTCGGGTCGGCGT-GCTGTTGTTGCAA
Dioeco_ca  TGTCCGTGCGTGTTATCGGCTACTGGTTGTC--GTCGGGTCGGCGT-GCTGTTGTTGCAA
Duplici_I  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplic_mi  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplic_mi  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplic_mi  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplici_I  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplici_I  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplici_I  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplici_I  CATCACTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplic_ka  CATCTCTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplic_ka  CATCTCTGCGTGTTATCGGTTACTGCTTGTC--AACGGGCCTGCCTAGTGGTTGTTGTAA
Duplic_II  CATCTTTGCGTGATATCGGCTACTGCTTGTC--AACGAGCCTGCCTTGCAGTTGTTGTAA
Duplic_II  CATCTTTGCGTGATATCGGCTACTGCTTGTC--AACGAGCCTGCCTTGCAGTTGTTGTAA
Duplic_II  CATCTTTGCGTGATATCGGCTACTGCTTGTC--AACGAGCCTGCCTTGCAGTTGTTGTAA
Duplic_II  CATCTTTGCGTGATATCGGCTACTGCTTGTC--AACGAGCCTGCCTTGCAGTTGTTGTAA
Duplic_II  CATCTTTGCGTGATATCGGCTACTGCTTGTC--AACGAGCCTGCCTTGCAGTTGTTGTAA
Duplic_II  CATCTTTGCGTGATATCGGCTACTGCTTGTC--AACGAGCCTGCCTTGCAGTTGTTGTAA
Rhodo_pau  TATGTCTGCGTGTTATCGGCTACCGGTTGTCTTAACGGGCCTGCCTTACGATTGTCA-AG
Rhodo_pau  TATGTCTGCGTGTTATCGGCTACCGGTTGTCTTAACGGGCCTGCCTTACGATTGTCA-AG
Rhodo_lar  TATGTCTGCGTGTTATCGGCTACCGGTTGTCTTAACGGGCCTGCCTTACGATTGTCA-AG
Rhodo_lar  TATGTCTGCGTGTTATCGGCTACCGGTTGTCTTAACGGGCCTGCCTTACGATTGTCA-AG
Rhodo_lar  TATGTCTGCGTGTTATCGGCTACCGGTTGTCTTAACGGGCCTGCCTTACGATTGTCA-AG
Rhodo_lar  TATGTCTGCGTGTTATCGGCTACCGGTTGTCTTAACGGGCCTGCCTTACGATTGTCA-AG
Rhine_sp   TGTGTTCGCGTGTTATCGGCTGCTGCTTGTCTTATCGGGCCTGCTTGGCAATTGTTGTAG
Echen_ma   TGTGCCGACGTGTTATCGGCTACTAGTCGCTTTATCGGGCCTGCTTAATGCATGTTATAG

           780       790       800       810       820       830
Dioeco_ca  CTGC-TGCGTGCCGGCGCGGTGGGCTATGGCGGTATGAA--ACTGTGCAAGGCACCGGAG
Dioeco_ca  CTGC-TGCGTGCCGGCGCGGTGGGCTATGGCGGTATGAA--ACTGTGCAAGGCACCGGAG
Duplici_I  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplic_mi  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplic_mi  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplic_mi  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplici_I  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplici_I  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplici_I  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplici_I  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplic_ka  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplic_ka  TCGC-CGCTGGGTGGCTTGGTGGACTACAGTGGTAAGAATAACTGTGCAAGGCACCGGGG
Duplic_II  TCGC-TGCTTGGTGGCTTGGTGGACTACAGTGGTATGTATAACTGTGCAAGGCACCGGGG
Duplic_II  TCGC-TGCTTGGTGGCTTGGTGGACTACAGTGGTATGTATAACTGTGCAAGGCACCGGGG
Duplic_II  TCGC-TGCTTGGTGGCTTGGTGGACTACAGTGGTATGTATAACTGTGCAAGGCACCGGGG
Duplic_II  TCGC-TGCTTGGTGGCTTGGTGGACTACAGTGGTATGTATAACTGTGCAAGGCACCGGGG
Duplic_II  TCGC-TGCTTGGTGGCTTGGTGGACTACAGTGGTATGTATAACTGTGCAAGGCACCGGGG
Duplic_II  TCGC-TGCTTGGTGGCTTGGTGGACTACAGTGGTATGTATAACTGTGCAAGGCACCGGGG
Rhodo_pau  CCGT-CGTAGGGTGGTCTGGTGGACCTTAGTGGTAAGAATAACTGTGCAGGGCACTGGAG
Rhodo_pau  CCGT-CGTAGGGTGGTCTGGTGGACCTTAGTGGTAAGAATAACTGTGCAGGGCACTGGAG
Rhodo_lar  CCGT-CGTAGGGTGGTCTGGTGGACCTTAGTGGTAAGAATAACTGTGCAGGGCACTGGAG
Rhodo_lar  CCGT-CGTAGGGTGGTCTGGTGGACCTTAGTGGTAAGAATAACTGTGCAGGGCACTGGAG
Rhodo_lar  CCGT-CGTAGGGTGGTCTGGTGGACCTTAGTGGTAAGAATAACTGTGCAGGGCACTGGAG
Rhodo_lar  CCGT-CGTAGGGTGGTCTGGTGGACCTTAGTGGTAAGAATAACTGTGCAGGGCACTGGAG
Rhine_sp   CCGT-TGTCAGGTGGTCTGGTGGGCCATGGTGGTATGAATAACTGTGCAGGGCATCGGAG
Echen_ma   CCGTGTGTTTGGTGGTCTGGTGGACTTTGATGGTAATAATAACTGTGCAGGGCACCGGAG
```

```
           840       850       860       870       880       890
Dioeco_ca  TTATCGGTCTCAAATGCATCTCGCACGCACGTTGTAAGTGC-------TGTGGGGTGCCG
Dioeco_ca  TTATCGGTCTCAAATGCATCTCGCACGCACGTTGTAAGTGC-------TGTGGGGTGCCG
Duplici_I  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplic_mi  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplic_mi  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplic_mi  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplici_I  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplici_I  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplici_I  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplici_I  ATGTCGGCCTCAATTGCACTCCGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplic_ka  ATGTCGGCCTCAATTGCACTCTGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplic_ka  ATGTCGGCCTCAATTGCACTCTGCATACACATTACAAGTGG-------TGTGGGGTGCCA
Duplic_II  ATTACGGCTTCAAATGCGCTCTGCATACACGTTACAAGTGG-------TGTGGGGTGCCA
Duplic_II  ATTACGGCTTCAAATGCGCTCTGCATACACGTTACAAGTGG-------TGTGGGGTGCCA
Duplic_II  ATTACGGCTTCAAATGCGCTCTGCATACACGTTACAAGTGG-------TGTGGGGTGCCA
Duplic_II  ATTACGGCTTCAAATGCGCTCTGCATACACGTTACAAGTGG-------TGTGGGGTGCCA
Duplic_II  ATTACGGCTTCAAATGCGCTCTGCATACACGTTACAAGTGG-------TGTGGGGTGCCA
Duplic_II  ATTACGGCTTCAAATGCGCTCTGCATACACGTTACAAGTGG-------TGTGGGGTGCCA
Rhodo_pau  TTTTCGGCCTCAAATGCAGCCAGCATGCACGCTTGTGGTGTCAA----TTGTGGGTGCTC
Rhodo_pau  TTTTCGGCCTCAAATGCAGCCAGCATGCACGCTTGTGGTGTCAA----TTGTGGGTGCTC
Rhodo_lar  TTTTCGGCCTCAAATGCAGCCAGCATGCACGCTTGTGGTGTCAA----TTGTGGGTGCTC
Rhodo_lar  TTTTCGGCCTCAAATGCAGCCAGCATGCACGCTTGTGGTGTCAA----TTGTGGGTGCTC
Rhodo_lar  TTTTCGGCCTCAAATGCAGCCAGCATGCACGCTTGTGGTGTCAA----TTGTGGGTGCTC
Rhodo_lar  TTTTCGGCCTCAAATGCAGCCAGCATGCACGCTTGTGGTGTCAA----TTGTGGGTGCTC
Rhine_sp   TTGTCGGCCTCAAATGCAGTTGGCATGCACGCTACAAGTGTGCGCG--TAGTGGGTTGCG
Echen_ma   TTTTCGGCCTCAAATGCAGTCTGCATGCGTGCTATGAGTGTAATGTGTTTGTGGGTGTCG

           900       910       920       930       940       950
Dioeco_ca  TTGCTGTTTGCTCACCGTTTGGTCTGATTGTCGTGTTGCCTGCGCAAAGTAGGTCCGGCA
Dioeco_ca  TTGCTGTTTGCTCACCGTTTGGTCTGATTGTCGTGTTGCCTGCGCAAAGTAGGTCCGGCA
Duplici_I  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGCA
Duplic_mi  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGCA
Duplic_mi  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGCA
Duplic_mi  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGCA
Duplici_I  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGCA
Duplici_I  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGCA
Duplici_I  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGCA
Duplici_I  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTCGCCTGCACAAAGTAGGTCCGGNA
Duplic_ka  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCACAAAGTAGGTCCGGCA
Duplic_ka  TCGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCACAAAGTAGGTCCGGCA
Duplic_II  TTGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCAAAAAGTAGGCCCGGCA
Duplic_II  TTGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCAAAAAGTAGGCCCGGCA
Duplic_II  TTGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCAAAAAGTAGGCCCGGCA
Duplic_II  TTGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCAAAAAGTAGGCCCGGCA
Duplic_II  TTGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCAAAAAGTAGGCCCGGCA
Duplic_II  TTGCTGCCTGCTTACTGTTGAGTCTGATTGTCGTGTTGCCTGCAAAAAGTAGGCCCGGCA
Rhodo_pau  TCGTGGCCTGCCTGTTATCGGGTCTGATTGTCGTGTTGCCTGCGCAAAGTGGGTTCGGCA
Rhodo_pau  TCGTGGCCTGCCTGTTATCGGGTCTGATTGTCGTGTTGCCTGCGCAAAGTGGGTTCGGCA
Rhodo_lar  TCGTGGCCTGCCTGTTATCGGGTCTGATTGTCGTGTTGCCTGCGCAAAGTGGGTTCGGCA
Rhodo_lar  TCGTGGCCTGCCTGTTATCGGGTCTGATTGTCGTGTTGCCTGCGCAAAGTGGGTTCGGCA
Rhodo_lar  TCGTGGNCTGCCTGTTATCGGGTCTGATTGTCGTGTTGCCTGCGCAAAGTGGGTTCGGCA
Rhodo_lar  TCGTGGCCTGCCTGTTATCGGGTCTGATTGTCGTGTTGCCTGCGCAAAGTGGGTTCGGCA
Rhine_sp   TTGCAGCCTGCCTGTTGTTGGGTGTGATTGTCGTGTTGCCTGCGCAAAGTGGGCCCGGCA
Echen_ma   TCGCGAGCTGGCTGTTGTTGGATTTGATTGTCGTGTTGCCTGCGAAAAGTGGGCCCGGCA
```

```
           960       970       980       990      1000       1010
Dioeco_ca  ATGGTTTAATGTGAGTTAG--ACAGTGGATGGTGCCTCGTGTGTG--AGTTGTGGTGGGC
Dioeco_ca  ATGGTTTAATGTGAGTTAG--ACAGTGGATGGTGCCTCGTGTGTG--AGTTGTGGTGGGC
Duplici_I  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplic_mi  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplic_mi  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplic_mi  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplici_I  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplici_I  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplici_I  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplici_I  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTCCGCGTGTA--GAGTGTGGTGGGC
Duplic_ka  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Duplic_ka  GTGGCTTGATGTAAGGTGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Duplic_II  GTGGCTTAATGTGAGATGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Duplic_II  GTGGCTTAATGTGAGATGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Duplic_II  GTGGCTTAATGTGAGATGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Duplic_II  GTGGCTTAATGTGAGATGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Duplic_II  GTGGCTTAATGTGAGATGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Duplic_II  GTGGCTTAATGTGAGATGG--GTAGTTGGTGGTGCTTCGCGTGCA--GGGTGTTGTGGGC
Rhodo_pau  GTGGCTCGGTGCAGGTTGGGTCTCGTGGG--GTGTCTACAGTGTT--GGTTGTAGTGGGC
Rhodo_pau  GTGGCTCGGTGCAGGTTGGGTCTCGTGGG--GTGTCTACAGTGTT--GGTTGTAGTGGGC
Rhodo_lar  GTGGCTCGGTGCAGGTTGGGTCTCGTGGG--GTGTCTACAGTGTT--GGTTGTAGTGGGC
Rhodo_lar  GTGGCTCGGTGCAGGTTGGGTCTCGTGGG--GTGTCTACAGTGTT--GGTTGTAGTGGGC
Rhodo_lar  GTGGCTCGGTGCAGGTTGGGTCTCGTGGG--GTGTCTACAGTGTT--GGTTGTAGTGGGC
Rhodo_lar  GTGGCTCGGTGCAGGTTGGGTCTCGTGGG--GTGTCTACAGTGTT--GGTTGTAGTGGGC
Rhine_sp   GTGGCTCGCGACAGGTCGG-AGTTGTTGGCGTAGTCTGCTGTGTGTCAGCTGTGGAGGGC
Echen_ma   GTGGTTCGATACAGATTGG-TCTCGTTGGCGGTGCCTGCGATGTA--GACTGTGGTGGGC

           1020      1030      1040       1050     1060       1070
Dioeco_ca  CAAATAATCGGTGGTGCTGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Dioeco_ca  CAAATAATCGGTGGTGCTGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplici_I  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_mi  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_mi  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_mi  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplici_I  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplici_I  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplici_I  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplici_I  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_ka  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_ka  CAAGTAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_II  CAAATAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_II  CAAATAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_II  CAAATAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_II  CAAATAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_II  CAAATAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Duplic_II  CAAATAATCAGTGGTGCAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Rhodo_pau  CTGATAGTCAGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Rhodo_pau  CTGATAGTCAGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Rhodo_lar  CTGATAGTCAGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Rhodo_lar  CTGATAGTCAGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Rhodo_lar  CTGATAGTCAGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Rhodo_lar  CTGATAGTCAGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Rhine_sp   CTGATAGTCGGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
Echen_ma   CAGATAGTCTGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCTTGAAACACGGACCAA
```

```
           1080      1090      1100       1110     1120       1130
Dioeco_ca  GGAGTTCAACATGTATGCGAGTCACTGGGTC------CTACGAAACCCAAAGGCGCAATG
Dioeco_ca  GGAGTTCAACATGTATGCGAGTCACTGGGTC------CTACGAAACCCAAAGGCGCAATG
Duplici_I  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplic_mi  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplic_mi  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplic_mi  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplici_I  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplici_I  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplici_I  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplici_I  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplic_ka  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplic_ka  GGAGTTCAACATGTATGCGAGTCAATGGGTC------CTACGAAACCCAAAGGCGCAGTG
Duplic_II  GGAGTTCAACATGTATGCGAGTCAATGGGCC------CTACGAAACCCAAAGGCGCAATG
Duplic_II  GGAGTTCAACATGTATGCGAGTCAATGGGCC------CTACGAAACCCAAAGGCGCAATG
Duplic_II  GGAGTTCAACATGTATGCGAGTCAATGGGCC------CTACGAAACCCAAAGGCGCAATG
Duplic_II  GGAGTTCAACATGTATGCGAGTCAATGGGCC------CTACGAAACCCAAAGGCGCAATG
Duplic_II  GGAGTTCAACATGTATGCGAGTCAATGGGCC------CTACGAAACCCAAAGGCGCAATG
Duplic_II  GGAGTTCAACATGTATGCGAGTCAATGGGCC------CTACGAAACCCAAAGGCGCAATG
Duplic_II  GGAGTTCAACATGTATGCGAGTCAATGGGCC------CTACGAAACCCAAAGGCGCAATG
Rhodo_pau  GGAGTTTAACATGTGTGCAAGTCATGGGGTC------TTACGAAACCCAAAGGCGTAGTG
Rhodo_pau  GGAGTTTAACATGTGTGCAAGTCATGGGGTC------TTACGAAACCCAAAGGCGTAGTG
Rhodo_lar  GGAGTTTAACATGTGTGCAAGTCATGGGGTC------TTACGAAACCCAAAGGCGTAGTG
Rhodo_lar  GGAGTTTAACATGTGTGCAAGTCATGGGGTC------TTACGAAACCCAAAGGCGTAGTG
Rhodo_lar  GGAGTTTAACATGTGTGCAAGTCATGGGGTC------TTACGAAACCCAAAGGCGTAGTG
Rhodo_lar  GGAGTTTAACATGTGTGCAAGTCATGGGGTC------TTACGAAACCCAAAGGCGTAGTG
Rhine_sp   GGAGTTTAACATGTGTGCAAGTCATGGGGTGTTTTTACTACGAAACCCAAAGGCGCAGTG
Echen_ma   GGAGTTTAACATGTGTGCAAGTCGTAGGGTC------CTACGAAACCCGAAGGCGCAGTG

           1140      1150      1160       1170     1180       1190
Dioeco_ca  AAAGTGAAGCTTCGACATGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Dioeco_ca  AAAGTGAAGCTTCGACATGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Duplici_I  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplic_mi  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplic_mi  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplic_mi  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplici_I  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplici_I  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplici_I  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplici_I  AAAGTAAAGCTTCGACATGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-A-
Duplic_ka  AAAGTAAAGCTTCGACTTGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-AC
Duplic_ka  AAAGTAAAGCTTCGACTTGTTCTCGGAG----------TGAGGTGAGATCCTGCCGT-AC
Duplic_II  AAAGTAAAGCTTCGACTTGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Duplic_II  AAAGTAAAGCTTCGACTTGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Duplic_II  AAAGTAAAGCTTCGACTTGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Duplic_II  AAAGTAAAGCTTCGACTTGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Duplic_II  AAAGTAAAGCTTCGACTTGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Duplic_II  AAAGTAAAGCTTCGACTTGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Duplic_II  AAAGTAAAGCTTCGACTTGTTCTCGAAG----------TGAGGTGAGATCCTGCCGTTAC
Rhodo_pau  AAAGTGAAGCTTCGTGTCCCT--CGGGG---CATGGAGTGAGGTGAGATCCCGTCGTTTC
Rhodo_pau  AAAGTGAAGCTTCGTGTCCCT--CGGGG---CATGGAGTGAGGTGAGATCCCGTCGTTTC
Rhodo_lar  AAAGTGAAGCTTCGTGTCCCT--CGGGG---CATGGAGTGAGGTGAGATCCCGTCGTTTC
Rhodo_lar  AAAGTGAAGCTTCGTGTCCCT--CGGGG---CATGGAGTGAGGTGAGATCCCGTCGTTTC
Rhodo_lar  AAAGTGAAGCTTCGTGTCCCT--CGGGG---CATGGAGTGAGGTGAGATCCCGTCGTTTC
Rhodo_lar  AAAGTGAAGCTTCGTGTCCCT--CGGGG---CATGGAGTGAGGTGAGATCCCGTCGTTTC
Rhine_sp   AAAGTGAGGCCTCAATT------CGAGG---TATT------GGTGAGATCCTGTCGTTAC
Echen_ma   AAAGTAAAGCTTTGGCTTCCTTTCGGGGATGTCTGAAGTGAGGTGAGATCCTGTCGTTAC
```

```
        1200      1210      1220       1230      1240      1250
Dioeco_ca  TCGCACCAGCCCG----TGTCAGCAACAGGCTAAG---AGCGGTAGGCGCATCACCGGCC
Dioeco_ca  TCGCACCAGCCCG----TGTCAGCAACAGGCTAAG---AGCGGTAGGCGCATCACCGGCC
Duplici_I  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_mi  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_mi  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_mi  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplici_I  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplici_I  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplici_I  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplici_I  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_ka  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_ka  TCGCATCAGCCTA------------TCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_II  TCGCATCAGCCTAA---TGCCAGCATCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_II  TCGCATCAGCCTAA---TGCCAGCATCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_II  TCGCATCAGCCTAA---TGCCAGCATCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_II  TCGCATCAGCCTAA---TGCCAGCATCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_II  TCGCATCAGCCTAA---TGCCAGCATCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_II  TCGCATCAGCCTAA---TGCCAGCATCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Duplic_II  TCGCATCAGCCTAA---TGCCAGCATCAGGCTAAG---AGCGGTGGGCGCATCACCGGCC
Rhodo_pau  TC-CGCCAGTCTCTTCG-GTCAGAAGCAGGCATTA--GAGCGGCGGGCGCATCACCGGCC
Rhodo_pau  TC-CGCCAGTCTCTTCG-GTCAGAAGCAGGCATTA--GAGCGGCGGGCGCATCACCGGCC
Rhodo_lar  TC-CGCCAGTCTCTTCG-GTCAGAAGCAGGCATTA--GAGCGGCGGGCGCATCACCGGCC
Rhodo_lar  TC-CGCCAGTCTCTTCG-GTCAGAAGCAGGCATTA--GAGCGGCGGGCGCATCACCGGCC
Rhodo_lar  TC-CGCCAGTCTCTTCG-GTCAGAAGCAGGCATTA--GAGCGGCGGGCGCATCACCGGCC
Rhodo_lar  TC-CGCCAGTCTCTTCG-GTCAGAAGCAGGCATTA--GAGCGGCGGGCGCATCACCGGCC
Rhine_sp   TTGCACCGCTTTTGCTGTGTCAGCAGCAGGCAAAGAAGAGCGGCAGGCGCATCACCGGCC
Echen_ma   TCGCATCAGCTT------GCCAGTGGTAGGCATCAA-GAGCGGCAGGCGCATCACCGGCC


           1260              1283
Dioeco_ca  CGTCCCATGATGTGGTCATTGG
Dioeco_ca  CGTCCCATGATGTGGTCATTGG
Duplici_I  CGTCCCATGATGTGGCCGCTGG
Duplic_mi  CGTCCCATGATGTGGCCGCTGG
Duplic_mi  CGTCCCATGATGTGGCCGCTGG
Duplic_mi  CGTCCCATGATGTGGCCGCTGG
Duplici_I  CGTCCCATGATGTGGCCGCTGG
Duplici_I  CGTCCCATGATGTGGCCGCTGG
Duplici_I  CGTCCCATGATGTGGCCGCTGG
Duplici_I  CGTCCCATGATGTGGCCGCTGG
Duplic_ka  CGTCCCATGATGTGGCCGCTGG
Duplic_ka  CGTCCCATGATGTGGCCGCTGG
Duplic_II  CGTCCCATGATGTGGCCATTGG
Duplic_II  CGTCCCATGATGTGGCCATTGG
Duplic_II  CGTCCCATGATGTGGCCATTGG
Duplic_II  CGTCCCATGATGTGGCCATTGG
Duplic_II  CGTCCCATGATGTGGCCATTGG
Duplic_II  CGTCCCATGATGTGGCCATTGG
Duplic_II  CGTCCCATGATGTGGCCATTGG
Rhodo_pau  CGTCCCATGATGTGGTCATTGG
Rhodo_pau  CGTCCCATGATGTGGTCATTGG
Rhodo_lar  CGTCCCATGATGTGGTCATTGG
Rhodo_lar  CGTCCCATGATGTGGTCATTGG
Rhodo_lar  CGTCCCATGATGTGGTCATTGG
Rhodo_lar  CGTCCCATGATGTGGTCANNNN
Rhine_sp   CGTCCCATGATGTGGTCATTGG
Echen_ma   CGTCCCATAATGTGGTCATTGG
```

Abbreviations:
Dioeco_ca = *Dioecotaenia cancellata*; Duplici_I = *Duplicibothrium* larva typeI; Duplici_II = *Duplicibothirum* larva type II; Duplic_mi = *Duplicibothrium minutum*; Duplic_ka = *Duplicibothrium karenae*; Rhodo_lar = *Rhodobothrium* larva; Rhodo_pau = *Rhodobothrium paucitesticulare*; Rhine_sp = *Rhinebothrium* sp.; Echen_ma = *Echencibothrium maculatum*.